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(71) Applicant *(for all designated States except US)*: ASTRAZENECA AB [SE/SE]; S-151 85 Södertälje (SE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BELFIELD, Graham [GB/GB]; AstraZeneca R & D Charnwood, Bakewell Road, Loughborough, Leics. LE11 5RH (GB). OAKLEY, Caroline [GB/GB]; AstraZeneca R & D Charnwood, Bakewell Road, Loughborough, Leics. LE11 5RH (GB).

(74) Agent: ASTRAZENECA AB; Global Intellectual Property, S-151 85 Södertälje (SE).

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(54) Title: COMPOSITIONS AND METHODS UTILIZING SEQUENCES FOR CONTROLLING NUCLEIC ACID EXPRESSION IN YEAST

(57) Abstract: The invention provides novel yeast promoters useful for controlling the expression of homologous and heterologous nucleic acid molecules in yeast cells. The yeast promoters are induced by a fermentable carbon source, such as glucose, or a non-fermentable carbon source, such as ethanol, or both. Therefore, expression of nucleic acid molecules encoding a polypeptide under the control of the novel yeast promoters may be regulated by varying the level of a fermentable carbon source, or a non-fermentable carbon source, or both.

COMPOSITIONS AND METHODS UTILIZING SEQUENCES FOR CONTROLLING NUCLEIC ACID EXPRESSION IN YEAST

BACKGROUND OF THE INVENTION

The controlled production in yeast of an enormous variety of useful proteins or polypeptides can be achieved using recombinant DNA technology. Yeast cells can be transformed with yeast expression vectors, which contain homologous or heterologous nucleic acid molecules encoding polypeptides (coding sequences). The yeast cells can then produce large quantities of the useful proteins or polypeptides in yeast cell culture.

Expression of the nucleic acid molecule encoding a polypeptide by the yeast expression vector is initiated at a region known as the promoter, which is recognized by and bound by RNA polymerase. The RNA polymerase travels along the DNA, transcribing the information contained in the coding strand from its 5' to 3' end into messenger RNA, which is in turn translated into a polypeptide having the amino acid sequence for which the DNA codes. The present invention provides novel yeast promoters useful for, *inter alia*, controlling the expression of homologous and heterologous nucleic acid sequences encoding proteins and polypeptides in yeast cells.

SUMMARY OF THE INVENTION

It is an object of the invention to provide novel yeast promoters, yeast expression vectors, and transformed yeast cells. It is a further object of the invention to provide a method for producing proteins and polypeptides in yeast cell culture.

In one embodiment of the invention a yeast promoter which comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide is provided. The promoter sequences are shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

The promoter is operative when operably linked to a nucleic acid molecule encoding a polypeptide.

As used herein, the term Apromoter@ refers to a nucleic acid sequence which is cable of initiating transcription of a nucleic acid molecule encoding a polypeptide (coding sequence); a Ayeast promoter@ is capable of initiating transcript of a coding sequence in yeast cells; and Apromoter activity@ refers to the level or amount of transcription initiation of a coding sequence, and encompasses any level above background (*i.e.*, the level or amount that occurs in the absence of a promoter; a background level, which is normally zero).

Another embodiment of the invention provides a yeast promoter which comprises an isolated and purified polynucleotide. The promoter sequences are shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4. The promoter is operative when operably linked to a nucleic acid molecule encoding a polypeptide.

Yet another embodiment of the invention provides a yeast promoter fragment which comprises at least 17 contiguous nucleotides of a polynucleotide. The polynucleotides are shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4. The fragment has promoter activity as determined by cloning the fragment into a yeast expression vector, wherein the fragment is operably linked to a reporter gene, transforming yeast cells with the yeast expression vector, growing the yeast cells in yeast cell culture under conditions favorable for expression of the reporter gene, and assaying the yeast culture for a reporter protein expressed by the reporter gene. The expression of the reporter gene indicates the fragment has promoter activity.

Still another embodiment of the invention provides a yeast expression vector comprising a yeast promoter. The promoter sequences are shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4. The promoter is operative when operably linked to a nucleic acid molecule encoding a polypeptide.

A further embodiment of the invention provides a yeast expression vector where activity of the promoter is controlled by varying the level of a non-fermentable carbon source, such as ethanol, in a medium of yeast cells in culture. The yeast cells are transformed with said yeast expression vector.

In yet another embodiment of the invention, a yeast expression vector comprising a yeast promoter which comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide is provided. The promoter sequences are shown in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:4. Promoter activity is controlled by varying the level of a fermentable carbon source in a medium of yeast cells in culture, where the yeast cells are transformed with the yeast expression vector. The fermentable carbon source can be glucose.

Another embodiment of the invention provides a yeast expression vector comprising a yeast promoter. The yeast promoter comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide. The promoter sequences are shown in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:4. Promoter activity is controlled by varying the level of a fermentable carbon source and a non-fermentable carbon source, such as ethanol, in a medium of yeast cells in culture, where the yeast cells are transformed with the yeast

expression vector. The fermentable carbon source can be glucose. The non-fermentable carbon source can be ethanol.

Still another embodiment of the invention provides a yeast cell transformed with a yeast expression vector. The yeast expression vector comprises a yeast promoter. The promoter sequences are shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4. The promoter is operative when operably linked to a nucleic acid molecule encoding a polypeptide.

Yet another embodiment of the invention provides a method for producing a polypeptide. A yeast expression vector is constructed where a polynucleotide encoding the polypeptide is controlled by a yeast promoter. The yeast promoter comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide. The promoter sequences are shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4. The promoter is operative when operably linked to a nucleic acid molecule encoding a polypeptide. A culture of yeast cells is transformed with the yeast expression vector. The yeast cells are maintained in culture so that the polypeptide is expressed. The polypeptide is then recovered.

Still another embodiment of the invention provides a method for producing a polypeptide. A nucleic acid molecule encoding the polypeptide is cloned into an expression vector selected from the group consisting of pYLR110P+luc, pYMR251AP+luc, pYMR107P+luc, pZEO1P+luc, pYLR110P, pYMR251AP, pYMR107P, and pZEO1P. The nucleotide acid molecule is operably linked to a promoter of the expression vector. A culture

of yeast cells is transformed with the yeast expression vector. The yeast cells are maintained in culture so that the polypeptide is expressed and the polypeptide is then recovered.

Another embodiment of the invention provides a method for producing a polypeptide. A yeast expression vector is constructed where a nucleic acid molecule encoding the polypeptide is controlled by a yeast promoter. The yeast promoter comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide. The promoter sequences are shown in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:4. Yeast cells are transformed with the yeast expression vector and are maintained in culture medium. The expression of the nucleic acid molecule encoding the polypeptide is controlled by varying the level of a fermentable carbon source, such as glucose, in the culture medium. The polypeptide is then recovered.

Still another embodiment of the invention provides a method for producing a polypeptide. A yeast expression vector is constructed where a nucleic acid molecule encoding the polypeptide is controlled by a yeast promoter. The yeast promoter comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide. The promoter sequences are shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4. The promoter is operative when operably linked to a nucleic acid molecule. A culture of yeast cells is transformed with the yeast expression vector. The yeast cells are maintained in culture medium and the expression of the nucleic acid molecule encoding the polypeptide is controlled by varying the level of a non-fermentable carbons source, such as ethanol, in the culture medium. The polypeptide is then recovered.

Another embodiment of the invention provides a method for producing a polypeptide. A yeast expression vector is constructed where a nucleic acid molecule encoding the polypeptide is controlled by a yeast promoter. The yeast promoter comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide. The promoter sequences are shown in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:4. A culture of yeast cells is transformed with the yeast expression vector. The yeast cells are maintained in culture medium and the expression of the nucleic acid encoding the polypeptide is controlled by varying the level of a fermentable carbon source, such as glucose, and a non-fermentable carbon source, such as ethanol, in the culture medium. The polypeptide is then recovered.

Yet another embodiment of the invention provides a method of identifying a promoter fragment with promoter activity by generating a fragment comprising at least 17 contiguous nucleotides of an isolated and purified polynucleotide. The polynucleotides are shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4. The fragment is cloned into a yeast expression vector, so that the fragment is operably linked to a reporter gene. Yeast cells are transformed with the yeast expression vector and grown in yeast cell culture under conditions favorable for expression of the reporter gene. The yeast culture is assayed for a reporter protein expressed by the reporter gene. Expression of the reporter gene indicates the fragment has promoter activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a map of YEp13 expression vector.

Figure 2 schematically illustrates construction of YLR110C and YMR251WA promoter constructs.

Figure 3 is a map of pPRB1P.

Figure 4 is a map of pPRB1P+luc.

Figure 5 is a map of pYLR110P+luc.

Figure 6 is a is a map of pYMR251AP+luc.

Figure 7 is a map of pYMR107P+luc.

Figure 8 is a map of pZEO1P+luc.

Figure 9 is a map pYLR110P.

Figure 10 is a map of pYMR251AP.

Figure 11 is a map of pYMR107P.

Figure 12 is a map of pZEO1P.

Figure 13 schematically illustrates the YLR110C promoter region.

Figure 14 schematically illustrates the YMR251WA promoter region.

Figure 15 schematically illustrates the YMR107W promoter region.

Figure 16 schematically illustrates the ZEO1 promoter region.

DETAILED DESCRIPTION OF THE INVENTION

Novel yeast promoters whose activity can be controlled by a fermentable carbon source, such as glucose, or a non-fermentable carbon source, such as ethanol, or both have been identified. The yeast promoters are useful for, *inter alia*, the high level production of proteins or polypeptides in yeast cell culture.

Yeast Promoters

The isolated and purified promoter polynucleotides of the invention are shown in SEQ ID NO:1 (the YLR110C promoter), SEQ ID NO:2 (the YMR251WA promoter), SEQ ID NO:3 (the YMR107W promoter), and SEQ ID NO:4 (the ZEO1 promoter). Yeast promoters comprising as little as 17 nucleic acids have been determined to function as promoters. The yeast promoters of the invention comprise at least 17, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600 or 700 contiguous nucleic acids of an isolated and purified polynucleotide up to the maximum length provided in any one of the sequences presented herein, that is, SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

Preferably, the promoter polynucleotides are isolated free of other components, such as proteins and lipids. The polynucleotides can be made by a cell and isolated or can be synthesized in the laboratory, for example, using an automatic synthesizer or an amplification method such as PCR.

Naturally occurring variants and artificial sequence variants (that is, those which do not occur in nature) of the promoters are included in the invention. Variants of the promoters and/or fragments thereof have, along their entire length, sequence identity of at least 90%,

and preferably greater than 95% as determined by the Smith-Waterman homology search algorithm as implemented in MPsrch™ program (University of Edinburgh) using an affine gap search with the following search parameters: gap open penalty: 12, gap extension penalty: 1.

Fragments of the full-length promoters are also functional as promoters. A promoter fragment of at least 17 contiguous nucleotides may occur at any position along the full-length promoter as shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4. Accordingly, promoter activity of 17 or more contiguous nucleotides occurring anywhere along the full-length promoter can be analyzed. Fragments of 17, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650 or 700, nucleotides of the promoters may be constructed by, for example, subjecting an isolated promoter to restriction endonucleases, to 5'- or 3'-deletion mutagenesis, to PCR, or to site specific deletion. A combination of these methods can also be used to generate fragments of a promoter.

The invention further embodies a hybrid promoter, *i.e.*, a promoter that comprises more than one promoter or more than one fragment of a promoter from which it was derived. The promoter fragments can be derived from more than one of the promoter sequences shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4. The promoters and fragments can be constructed as described above, ligated together, and cloned into a yeast expression vector. Where a promoter comprises nucleotides from at least two polynucleotides selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, at least 5, 6, 7, 8, 9, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350,

400, 450, 500, 550, 600, or 650 contiguous nucleotides are derived from each of the polynucleotides to form a promoter of at least 17 nucleotides. Alternatively, each of the full-length promoters can be combined with another full-length promoter or with fragments of another promoter.

The yeast promoters, fragments of the promoters, and hybrid promoters are useful for controlling expression of a protein or polypeptide when the yeast promoter is operably linked to a nucleic acid molecule encoding the protein or polypeptide.

Determination of Promoter Activity

Promoters and fragments of promoters can be assayed for promoter activity by cloning a fragment of a promoter, or a full-length promoter, or a hybrid promoter into a yeast expression vector so that is operably linked to a reporter gene, *i.e.*, a coding sequence for a reporter protein. The yeast expression vector is transformed in yeast cells, which are grown in yeast cell culture under conditions favorable for expression of the reporter gene, for example, under conditions providing a fermentable and/or non-fermentable carbon source. Expression of the reporter gene, as determined by an assay for the amount of a reporter protein expressed by the reporter gene, indicates that the promoter has activity.

For example, to determine if a promoter has activity, *i.e.* is operative, expression of a reporter gene by a promoter of the invention may be compared to expression of the reporter gene by a reference promoter such as PBR1 (Cottingham *et al.* (1991) Eur J Biochem 196(2):431-8; Sleep *et al.* (1991) Biotechnology 9(2):183-7; Finnis *et al.* (1992) Yeast 8(1):57-60; Meldgaard *et al.*(1995) Glycoconj J 12(3):380-90; Bach *et al.* (1996) Receptors

and Channels 4(2):129-39. A promoter, a fragment of a promoter, or a hybrid promoter of the invention is operative if it expresses at least 25% of the amount of a reporter protein as the full-length PBR1 promoter in a medium containing a non-fermentable carbon source, or a fermentable carbon source, or both. Preferably, an operative promoter expresses at least 50%, 75%,, 100%, 200%, 300%, 400%, or more of the amount of a reporter protein as the full-length PBR1 reference promoter.

Assays for promoter activity are useful for identifying yeast promoters with high activity and the specific nucleotide sequences of the promoters that are necessary for promoter activity.

Yeast Expression Vectors

The yeast promoters of the invention, which comprise isolated and purified polynucleotides selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4 or fragments thereof, can be used to construct yeast expression vectors.

Yeast expression vectors are any vectors capable of autonomous replication within a yeast host organism or capable of integrating into the yeast genome. Yeast expression vectors are useful for introducing foreign DNA into yeast cells. Typical yeast expression vectors include yeast integrative plasmids (YIp), yeast replicating plasmids (YRp), yeast expression plasmids (YXp), yeast centromere-containing plasmids (YCp), and yeast episomal plasmids (YEp). Preferably, a yeast expression vector can be selected and maintained in both yeast and *E. coli.*

Yeast expression vectors, typically plasmids, incorporate the yeast promoters of the invention to control expression of nucleic acid molecules encoding heterologous or homologous proteins or polypeptides. The nucleic acid molecules are operably linked to a promoter in the yeast expression vector. A wide range of heterologous eukaryotic and prokaryotic proteins or peptides may be expressed by the vectors of the invention.

Expression vectors incorporating the promoters can be constructed by inserting into a vector a nucleic acid molecule encoding a protein or polypeptide (coding sequence) which is to be expressed. The coding sequence can be inserted at a restriction site which is provided downstream of a translation start codon controlled by the promoter. The coding sequence must be inserted in the correct translational reading frame.

Alternatively, the polynucleotide can itself be provided with a translational start codon followed directly by a coding sequence. Where the promoter does not contain a translational start codon, a restriction site is provided so that the coding sequence can be inserted in the correct reading fame and so that its translational start codon is correctly positioned in relation to the promoter. The coding sequence can encode heterologous or homologous or eukaryotic or prokaryotic polypeptides or proteins. In a preferred embodiment the coding sequence encodes a fusion protein. The coding sequence may further comprise a signal sequence.

In addition to the promoters of the invention, other components can be added to the expression vectors of the invention. For example, yeast selective markers, such as *LEU2* or *TRP1*, which allow for selection of yeast cells that have been effectively transformed by the

vector can be added. A yeast replication origin, such as the replication origin of the 2-micron plasmid or the autonomous ARS replication segment can be added. Upstream activating sequences and transcription terminator sequences may be added. Further, at least a portion of a bacterial plasmid, such as found in YEp13, can be added to enable the yeast expression vector to be manipulated in an intermediate bacterial host system, such as *Escherichia coli.*

The expression vector may also comprise a reporter gene which encodes, for example, β-galactosidase or luciferase. The reporter gene can be under the control of a promoter of the invention. Where the reporter gene, *i.e.*, coding sequence, is linked to a gene encoding a desired protein, assaying the level of expression of the reporter protein can quickly and easily determine the level of expression of the desired protein.

The expression vectors of the invention can be used to direct the fermentable carbon source- and/or non-fermentable carbon source-induced high level expression of proteins or polypeptides in yeast. The promoters of the invention can be induced by the presence of a fermentable carbon source, such as glucose, or a non-fermentable carbon source, such as ethanol, or both. That is, the promoters have greater promoter activity in the presence of a fermentable carbon source, or a non-fermentable carbon source, or both than in the absence of a fermentable carbon source, or a non-fermentable carbon source, or both. Promoters YLR110C, as shown in SEQ ID NO:1; YMR251WA, as shown in SEQ ID NO:2; and ZEO1, as shown in SEQ ID NO:4, can be induced by a fermentable carbon source, such as glucose, or by a non-fermentable carbon source, such as ethanol, or by both. Promoter YMR107W, as shown in SEQ ID NO:3, can be induced by a non-fermentable carbon source, such as

ethanol. Thus, the amount of expression of a homologous or heterologous nucleic acid molecule encoding a protein operably linked to the promoters of the invention can be controlled by varying the amount of an available fermentable carbon source, such as glucose, or a non-fermentable carbon source, such as ethanol, or both.

Transformed Yeast Cells

Yeast cells can be transformed with the yeast expression vectors of the invention. Transformation can be accomplished by well known methods, including, but not limited to electroporation, calcium phosphate precipitation, and microinjection. The yeast expression vectors of the invention can be used to transform yeast cells, including, but not limited to *Saccharomyces cerevisiae, S. uvarum, S. carlsbergensis, Saccharomycopsis lipolytica, Schizosaccharomyces pombe*, and *Kluyveromyces lactis*.

Transformed yeast cells containing a yeast expression vector can be grown in an appropriate medium for the yeast. A fermentable or non-fermentable carbon source can be added to the yeast culture medium in order to control the activity of the promoter.

Methods of Production of Proteins

Yeast cells transformed with expression vectors comprising a promoter of the invention can be used to produce proteins and polypeptides. Under proper cell culture conditions, preferably in the presence of a fermentable or non-fermentable carbon source, or both, the promoters of the invention will control expression of a nucleic acid molecule encoding a polypeptide operably linked to the promoter.

The protein or polypeptide can be retained within the yeast cell. The yeast cells can be then harvested, lysed, and the protein obtained and substantially purified in accordance with conventional techniques. Such techniques include, but are not limited to chromatography, electrophoresis, extraction, and density gradient centrifugation.

In a preferred embodiment of the invention, the protein or polypeptide to be recovered will further comprise a signal peptide capable of transporting the protein or polypeptide through the membrane of a transformed yeast cell. The protein or polypeptide can be recovered from the culture medium by, for example, adsorption or precipitation.

Further, the proteins and polypeptides may be produced as a fusion protein, which includes not only the amino acid sequence of the desired protein, but also one or more additional proteins. Affinity purification protocols can be used to facilitate the isolation of fusion proteins. Typically, a ligand capable of binding with high specificity to an affinity matrix is chosen as the fusion partner for the desired protein. For example, fusion proteins made with glutathione-S-transferase can be selectively recovered on glutathione-agarose and IgG-Sepharose can be used to affinity purify fusion proteins containing staphylococcal protein A.

Preferably, the protein or polypeptide of interest can be separated from the remainder of the fusion protein. The fusion protein can be constructed so that a site for proteolytic or chemical cleavage is inserted between the protein of interest and the fusion partner. For example, sites for cleavage by collagenase, Factor Xa protease, thrombin, and enterokinase, have been inserted between the fusion partner and the protein of interest. The protein of

interest can be also cleaved from the remainder of the fusion protein by chemical cleavage by, for example, hydroxylamine, cyanogen bromide (CNBr), or N-chlorosuccinamide.

The following are provided for exemplification purposes only and are not intended to limit the scope of the invention described in broad terms above. All references cited in this disclosure are incorporated by reference.

EXAMPLE 1

Preparation of Yeast Samples

S. cerevisiae strain 11C

This example describes the growth of haploid *Saccharomyces cerevisiae* strain 11C. It has the genotype: *ade2*-161, *trp1*-Δ63, *ura3*-52, *lys2*-801, *leu2*Δ1 &/or *leu2*-3 &/or *leu2*-112, *his3*Δ200 &/or *his4*-519. 11C was generated by crossing the strains YPH500 (Mat a *ura3*-52 *lys2*-801 *ade2*-161 *trp1*-Δ63 *his3*Δ200 *leu2*Δ1) (Sikorski and Hieter. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122: 19-27) and AH22 (MATa *leu2*-3 *leu2*-112 *his4*-519) (Hinnen *et al.* (1978) Transformation of yeast. *Proc. Natl. Acad. Sci. USA* 75: 1929-1933).

Three sterile 500 ml conical flasks, each containing 100 ml sterile YPD broth (Sigma, Cat No. Y-1375) were inoculated with sterile 10µl loops of differing quantities of the *S. cerevisiae* strain 11C from a freshly streaked YPD plate (Sigma, Cat No. Y-1500), and grown in an orbital shaker at 30°C, 200 rpm, overnight. The growth of 11C in the three flasks was measured by absorbance at 600nm. One flask was deemed to be at the late

exponential growth phase (1.98 ODU ml at 600 nm), and this culture was used to inoculate (50ml o/n culture per flask) 2 identical 5L sterile conical flasks (labeled E and L), each containing 1L sterile YPD broth to a final concentration of ~0.1 ODU ml. Flasks E and L were grown in an orbital shaker at 30°C, 200 rpm. 10ml samples were collected at times indicated below (Table 1). The samples were treated as follows: their growth was determined (A600nm), the possibility of contamination was checked (using a light microscope), cells were harvested in a benchtop centrifuge (~2000xg for 5 minutes), and the supernatant removed and frozen at -20°C (samples labeled E0 - E3, and L0 - L5).

Table 1. *Growth of cultures E and L as measure by absorbance at 600 nm.*

Time Point	Time after inoculation (min)	Growth of flask E (ODU)	Growth of flask L (ODU)
T0	0	0.099	0.099
T1	310	0.37	0.36
T2	410	0.71	0.72
T3	455	0.97	0.92
T4	775	-	3.64
T5	1420	-	6.05

After 455 minutes, a time deemed to be late exponential growth phase in glucose, flask E (*i.e.* early) was harvested (~2000xg for 5 minutes), split into 50ml aliquots, and frozen at -80°C. After 1420 minutes, a time deemed to be growth on ethanol, flask L (*i.e.* late) was harvested (~2000xg for 5 minutes), split into 50ml aliquots, and frozen at -80°C.

Determination of Glucose and Ethanol concentration

Supernatant samples (E0 - E3, and L0 - L5) were defrosted, and their ethanol and glucose contents were measured using ethanol (Boehringer, Cat. No. 176290) and glucose (Boehringer, Cat. No. 176251) detection kits according to manufacturers instructions. The concentrations determined are shown below in Table 2.

Table 2. *Glucose and Ethanol concentrations in supernatants of cultures E and L at different time points.*

Sample	Time after inoculation (min)	Glucose level in media (g L^{-1})	Ethanol level in media (g L^{-1})
E0	0	20.0	0.0
E1	310	21.8	0.3
E2	410	21.8	0.8
E3	455	21.2	0.87
L0	0	20.0	0.0
L1	310	22.2	0.36
L2	410	22.0	0.62
L3	455	20.0	0.87
L4	775	11.8	5.2
L5	1420	0.0	11.8

It can seen in Table 2 that at the point of culture harvest for E (E3, 455 minutes), the cells were still utilizing glucose as a carbon source, while at the point of culture harvest for L (L5, 1420 minutes), glucose was exhausted, and the cells were utilizing ethanol as a carbon source. Calibration values used to calculate glucose concentrations are shown in Table 3. Calibration values used to calculate ethanol concentrations are shown in Table 4.

Table 3. *Glucose standards*

GLUCOSE STANDARDS g/l	OD A340
0	0
0.2	0.246
0.4	0.461
0.6	0.726
0.8	0.967
1	1.227

Table 4. *Ethanol standards*

ETHANOL STANDARDS g/L	OD A340
4.72	0.041
9.44	0.083
18.88	0.166
37.76	0.322
56.6	0.534
75.5	0.664
94.4	0.846

EXAMPLE 2

Analysis of RNA Levels From Yeast Dimorphic Growth Samples

Total RNA Isolation

Total RNA was isolated from 300ml of culture using the hot phenol protocol. The frozen yeast pellets were resuspended in lysis buffer (4ml) (0.5ml Tris-CL (1M, pH 7.5), 1.0 ml EDTA (0.5 M), 2.5ml 10% SDS, and 46.0ml ddH_2O) and an equal volume of acid phenol

was added and vortexed. Following incubation at 65°C for one hour (with occasional vigorous vortexing) the mixture was placed on ice for 10 minutes then centrifuged (10 minutes). The aqueous layer was transferred to a fresh centrifuge tube and mixed with an equal volume of phenol at room temperature. The mixture was centrifuged and an equal volume of chloroform was mixed with the aqueous layer in a fresh centrifuge tube. Following centrifugation the aqueous layer was transferred to a fresh centrifuge tube and sodium acetate (to a final concentration of 0.3M) and two volumes of 100% ethanol was added to precipitate the RNA. The mixture was placed at -20°C for 30 minutes then centrifuged for 10 minutes to pellet the RNA. The RNA pellet was washed 2-3 times with 70% ethanol then allowed to dry at room temperature. The pellet was resuspended in ddH2O (200-500 µL). The RNA was quantitated by measuring OD 260-280. Yield of total RNA was ~4.5mg from each culture.

Poly A+ RNA Purification

Poly A+ RNA was purified from total RNA using Qiagen Oligotex mRNA Midi Kit (Qiagen, Cat. No. 70042). 2mg of total RNA was used as starting material and made up to a volume of 500µl with DEPC treated H_2O. To this 500µl buffer OBB (2x binding buffer) and 55µl oligotex suspension was added. The AOligotex mRNA Spin-Column Protocol@ from the kit protocol booklet was followed. The pelleted mRNA was washed in 200µl 75% ethanol, dried and resuspended in 10µl DEPC treated H_2O. Yield of Poly A+ RNA was ~ 8µg for each sample.

cDNA Synthesis

cDNA was synthesized using the protocol for GeneChip Expression Analysis Manual using reagents from Gibco BRL Life Technologies Superscript Choice System cat. No. 18090-019. For each sample 5µg Poly A+ RNA was added to 100pmol of T7-$(dT)_{24}$ primer (sequence: GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG-(T)24, HPLC purified) (SEQ ID NO:15) in a total of 8µl (made up to volume with DEPC treated H_2O). The reaction mixture was incubated for 10 minutes at 70°C in a Perkin Elmer PE9600 thermalcycler then put on ice. The following reagents were added to the reaction mixture: 4µl 5x first strand cDNA buffer; 2µl 0.1M DTT; and 1µl 10mM dNTP mix. The reaction mixture was mixed and incubated at 37°C for 2 minutes in a Perkin Elmer PE9600 thermocycler. 5µl SuperScript II reverse transcriptase was then added. The mixture was incubated at 37°C for 1 hour in a Perkin Elmer PE9600 thermocycler.

The first strand cDNA reaction was placed on ice and the following reagents added: 91µl DEPC treated H_2O; 30µl 5x second strand reaction buffer; 3µl 10mM dNTP mix; 1µl 10units/µl *E. coli* DNA ligase; 4µl 10units/µl *E. coli* DNA Polymerase I; and 1µl 2units/µl RNase H. The mixture was incubated at 16°C for 2 hours in a Perkin Elmer PE9600 thermalcycler. 2µl 5units/µl T4 DNA Polymerase was then added. The mixture was incubated for a further 5 minutes at 16°C in a Perkin Elmer PE9600 thermalcycler. 10µl 0.5M EDTA was then added.

The double stranded DNA was cleaned up by phenol extraction. The reaction product transferred to a 1.5ml eppendorf tube and 162µl Tris pH 8.0 saturated phenol was

added. The tube was mixed by vortexing, the tube was then centrifuged in a microfuge at 13,000rpm for 5 minutes. The top fraction was recovered and cDNA precipitated by addition of 60µl 7.5M ammonium acetate plus 400µl absolute ethanol. This was immediately centrifuged in a microfuge at 13,000rpm for 20 minutes. The supernatant fraction was discarded, the pellet was washed in 75% ethanol and then air-dried. The pellet was resuspended in 20µl DEPC treated H_2O

Synthesis of Biotin-Labeled cRNA by In Vitro Transcription (IVT)

Reagents from Ambion MEGAscript T7 kit, cat. No. 1334, were used for the synthesis of biotin-labeled cRNA by *in vitro* transcription (IVT). The NTP Labeling mix comprised 7.5mM ATP; 7.5mM GTP; 5.625mM UTP; 1.875mM Biotin-16-UTP (Enzo cat No. 42814); 5.625mM CTP; and 1.875mM Biotin-11-CTP (Enzo cat No. 42818). The IVT Labeling reaction comprised: 14.5µl NTP Labeling mix; 2µl 10x Ambion Transcription Buffer; 1.5µl Double strand cDNA (from above); and 2µl Ambion T7 Enzyme Mix.

The reaction mixture was incubated for 6 hours at 37°C in a Perkin Elmer PE9600 thermalcycler. The biotinylated cRNA was cleaned up using Qiagen RNeasy kit, cat No. 74103. The RNeasy kit protocol was followed exactly. RNA was eluted in 2 aliquots of 30µl DEPC treated H_2O. The RNA was precipitated by addition of 6µl 3M sodium acetate pH 5.5 plus 75µl absolute ethanol. The RNA was allowed to precipitate overnight at -20°C. Samples were centrifuged in a microfuge at 13,000rpm for 20 minutes to pellet the RNA. The supernatant fraction was discarded and the pellet was washed in 1ml of 75% ethanol

and then allowed to air dry. The pellet was then resuspended in 20µl DEPC treated H_2O. The yield of cRNA was ~ 40µg for each sample.

cRNA Fragmentation

11µg of cRNA was fragmented. 8µl of 5x Fragmentation buffer (200mM Tris-Acetate pH 8.1, 500mM potassium acetate, 150mM magnesium acetate) plus 11µg cRNA made up to 20µl with DEPC treated H_2O was used. The reaction mixture was incubated 94°C for 35 minutes in a Perkin Elmer PE9600 thermal cycler.

Hybridization to GeneChip Microarray

The hybridization mix comprised: 20µl (11µg) of fragmented cRNA; 2.2µl of control oligo B2 (50pmol/µl) (sequence: 5=Biotin-GTCAAGATGCTACCGTTCAG 3= HPLC purified) (SEQ ID NO:16); 2.2µl Herring Sperm DNA (10mg/ml); 110µl 2x Buffer (2M NaCl, 20mM Tris pH 7.6, 0.01% Triton X-100); and 85.6µl DEPC treated H_2O. The hybridization mix heated to 95°C in a Techne hot block for 5 minutes, followed by incubation at 40°C for 5 minutes. The hybridization mix was clarified by centrifugation in microfuge at 13,000rpm for 5 minutes.

200µl of supernatant to added to the GeneChip cartridge (GeneChip cartridge was previously pre-wetted with 200µl 1x Buffer and incubated for 10 minutes at 40°C in the rotisserie box of a GeneChip hybridization oven 320 (cat No. 800127) at maximum rpm. The sample was hybridized to the microarray overnight at 40°C in a GeneChip hybridization oven in the rotisserie at maximum rpm.

Washing and Staining of Probe Arrays

The hybridization mix was recovered from the GeneChip cartridge and put back in the tube containing the remainder of the sample. 200µl 6x SSPE-T (6x SSPE plus 0.005% Triton X-100) was applied to the chip and pipetted in and out twice. This process was repeated twice more. Another 200µl 6x SSPE-T was applied to the cartridge and the cartridge was then incubated for 1 hour at 50°C at maximum rpm in the GeneChip hybridization oven. The 6x SSPE-T was removed and 200µl 0.5x SSPE-T was added to cartridge. The cartridge was incubated for 15 minutes at 50°C at maximum rpm in the GeneChip hybridization oven. The 0.5x SSPE-T was removed and the cartridge was re-filled with 200µl 6x SSPE-T.

The stain solution comprised: 190µl 6x SSPE-T; 10µl of 20mg/ml acetylated BSA; and 2µl 1mg/ml conjugated streptavidin:phycoerythrin (Molecular Probes cat. No. S-866). 200µl 6x SSPE-T was removed from the GeneChip cartridge and 200µl of stain solution added. The cartridge was incubated at ambient temperature in a GeneChip hybridization oven at maximum rpm in the rotisserie for 10 minutes. The stain solution was removed and the cartridge was washed by adding 200µl 6x SSPE-T and pipetting this in and out of the cartridge twice. This process was repeated six times. The cartridges were then completely filled with 6x SSPE-T and any bubbles removed. Hybridization, washing and staining was repeated using the same hybridization mixes until both samples had been hybridized to each of the four yeast chip sub-set arrays.

Data collection

Data was collected by scanning the hybridized chips on a Hewlett-Packard GeneArray scanner. A Ahalo@ effect (appearance of stain non-specifically across the array image) was seen on one of the scanned images: yeast growing in glucose rich media, sub-set C array. Scanning of this array was aborted after one scan and the chip was washed twice with 200µl 6x SSPE-T and then re-filled as before. This array was then re-scanned three times and the data collected was the average of these three scans. All other arrays were scanned four times without problems and the data collected was the average of the four scans.

EXAMPLE 3

Isolation of promoters and construction of expression vectors.

PCR amplification of promoter regions from genomic DNA

Based on the *Saccharomyces cerevisiae* genomic sequence in the GenEMBL nucleotide database oligonucleotide primers were designed to amplify the genomic sequence 5= to the following ORFs: YLR110C (Johnston *et al.* (1997) Nature 1997 May 29;387(6632 Suppl):87-90), YMR251WA (common name HOR7) (Bowman *et al.* (1997) Nature May 29;387(6632 Suppl):90-3), YMR107W (Bowman *et al.* (1997) Nature May 29;387(6632 Suppl):90-3), and YOL109W (common name ZEO1) (Dujon *et al.* (1997) Nature May 29;387(6632 Suppl):98-102). The region amplified was the non-coding region separating the selected ORF and the next predicted *Saccharomyces cerevisiae* ORF in the 5= direction, with a minimum length of 500bp.

Sequence of oligonucleotide primers used to amplify promoter DNA

HindIII, NheI and NdeI cloning sites underlined.

YLR110C-F	ATGCAAGCTTCGCGGCCGCCGTCTGATTTCCGTTT	SEQ ID NO:5
YLR110C-R	CCAGGCCGCATATGTCATATAGTGTTTAAG	SEQ ID NO:6
YMR251WA-F	AGCTAAGCTTCGCGGCCGCCTTTCGATTAGCACGCAC	SEQ ID NO:7
YMR251WA-R	AGATACCTTCATATGTTATTATTAGTC	SEQ ID NO:8
YMR107W-F	AGCTAAGCTTCGCGGCCGCGCAGAAATGATGAAGG	SEQ ID NO:9
YMR107W-R	ATCCATCCCATATGTGATATCTCGATTAG	SEQ ID NO:10
ZEO1-F	AGCTAAGCTTCGCGGCCGCGGAGGTCTGCTTCACG	SEQ ID NO:11
ZEO1-R	TACGATCGCATATGTAATTGATATAAACG	SEQ ID NO:12

PCR reactions were set up for each primer pair as follows: For YMR251WA and ZEO1 90µl of Reddy-Load PCR (1.1X) mix, 3.5mM $MgCl_2$ (Advanced Biotechnologies, cat.no. AB-0628); 2µl of forward primer (100µM); 2µl of reverse primer (100µM); 1µl of *S. cerevisiae* genomic DNA (Promega G310A, lot 8347702, 276µg/ml); and 5µl of H_2O were combined.

For YLR110C and YMR107W 90µl of Reddy-Load PCR (1.1X) mix, 1.5mM $MgCl_2$ (Advanced Biotechnologies, cat.no. AB-0575); 2µl of forward primer (100µM); 2µl of reverse primer (100µM); 1µl of *S. cerevisiae* genomic DNA (Promega G310A, lot 8347702, 276µg/ml); and 5µl of H_2O were combined.

The thermocycling was carried out as follows: For the YMR251WA promoter: 94°C for 5 minutes followed by 30 cycles of : 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 1 minute; followed by 72°C for 5 minutes. The reaction mixtures were then held at 4°C. For the YMR107W and ZEO1 promoters: 94°C for 5 minutes followed by 30 cycles of : 94°C for 30 seconds, 45°C for 30 seconds, 72°C for 1 minute; followed by 72°C for 5 minutes.

The reaction mixtures were then held at 4°C. For the YLR110C promoter: 94°C for 5 minutes followed by 30 cycles of : 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 1 minute; followed by 72°C for 5 minutes. The reaction mixtures were then held at 4°C.

The PCR solutions were loaded onto an LMP gel and the bands were purified using Wizard PCR Preps (Promega, cat. no. A7170) according to protocol, eluted in 50µl, ethanol precipitated, and resuspended in 20µl. A map of the YLR110C promoter region is shown in Figure 13 and SEQ ID NO:29. A map of the YMR251WA promoter region is shown in Figure 14 and SEQ ID NO:30. A map of the YMR107W promoter region is shown in Figure 15 and SEQ ID NO:31. A map of the ZEO1 promoter region is shown in Figure 16 and SEQ ID NO:32.

Cloning promoter regions into a yeast vector containing the luciferase gene

The PCR products representing the regions upstream of the YLR110C and YMR251WA ORFs were cloned into the suitably digested YEp13-based multicopy yeast expression vector pPRB1P+luc. A map of YEp13 is shown in Figure 1. The Accession number for YEp13 is U03498. A map of pPRB1P is shown in Figure 2. The sequence of pPRB1P is shown in SEQ ID NO:27. A map of pPRB1P+luc is shown in Figure 3 and the sequence is shown in SEQ ID NO:28. The PRB1 promoter was removed from the vector by digesting with the restriction enzymes HindIII and NdeI. The digested backbone was then ligated with a HindIII / NdeI digested PCR product. See Figure 4.

The PCR products described below, and maxi-prepped pPRB1P+luc were digested as follows. 60 µl of pPRBP1+luc (328µg/ml), 10 µl of Hind III (Life Technologies, cat.no.

15207-012, 10 units/µl), 10 µl NdeI (Amersham, cat.no. E0216Y, 20 units/µl), 10 µl NEBuffer 2 (NEB, cat.no. 007-2), and 10 µl of H_2O. 14 µl YLR110C, 2 µl of Hind III (Life Technologies, cat.no. 15207-012, 10 units/µl), 2 µl Nde I (Amersham, cat.no. E0216Y, 20 units/µl), and 2 µl NEBuffer 2 (NEB, cat.no. 007-2). 14 µl YMR251WA, 2 µl of Hind III (Life Technologies, cat.no. 15207-012, 10 units/µl), 2 µl Nde I (Amersham, cat.no. E0216Y, 20 units/µl), and 2 µl NEBuffer 2 (NEB, cat.no. 007-2). The solutions were allowed to react at 37°C, for 4 hours.

The double digested pPRB1P+luc backbone was purified on an LMP gel using Wizard PCR preps (Promega, cat. no. A7170), and then ethanol precipitated. The remaining digestion products were also ethanol precipitated. The pPBR1P+luc digests were resuspended in 60µl of H_2O and the PCR product digests were resuspended in 20µl.

Ligation reactions were then carried out between each promoter region and the digested pPRBP1+luc at 16°C overnight. The PCR products representing the regions upstream of the following ORFs; YMR107W and ZEO1, were prepared, restricted, and ligated essentially as described above, however BCL restriction buffer B and different amounts of PCR product/volumes were used.

Transformation of ligation products into E. coli

The products of the ligations described above were transformed into *E. coli* (Invitrogen=s One-Shot TOP10 Competent cells, cat.no. C4040-10) according to manufacturers protocol. In each case 5µl of the ligation product was added to the cell

suspension. The total final cell suspension was plated out onto L-amp plates and incubated overnight at 37°C.

Colonies were picked from the plates and PCR screened using the PCR primers used to amplify the promoters originally. Two positive colonies from each ligation were grown in 5ml overnight cultures and their plasmids were purified (Promega Wizard Plus SV Mini-preps, cat. no. A1330). The eluted DNA was ethanol precipitated and resuspended in 20µl of water. Analytical restriction digests were carried out to confirm the presence of the correct promoter. Clones containing all four promoter constructs were obtained.

The new constructs were named as follows:

pPRB1+luc backbone + YLR110C promoter = pYLR110P+luc	SEQ ID NO:19
pPRB1+luc backbone + YMR251WA promoter = pYMR251AP+luc	SEQ ID NO:20
pPRB1+luc backbone + YMR107W promoter = pYMR107P+luc	SEQ ID NO:21
pPRB1+luc backbone + ZEO1 promoter = pZEO1P+luc	SEQ ID NO:22

Maps of pYLR110P+luc, pYMR251AP+luc, pYMR107P+luc, and pZEO1P+luc are shown in Figures 5, 6, 7, and 8, respectively. Plasmid DNA (pYLR110P+luc and pYMR251AP+luc) was prepared for transformation into yeast and sequencing using the QIAGEN Plasmid Maxi kit (Cat.no. 12162). The DNA concentrations of the maxi-preps (measured by absorbance at 260 nm) were: pYLR110P+luc 463µg/ml; pYMR251AP+luc 346 µg/ml; pYMR107P+luc ~300µg/ml; and pZEO1P+luc ~720 µg/ml. The remaining plasmids were transformed into yeast as Wizard Plus SV Mini-prep DNA, and maxi-prep DNA was obtained for sequencing using the Gibco BRL Concert Plasmid Maxi kit (Cat no.11452).

Sequencing of promoter constructs

DNA of each of the four promoter constructs were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems, part no. 4303153) was used to carry out the sequencing reactions. Each reaction contained 8µl of Reaction Mix and 1µl of 3.2 µM primer. The volumes of template DNA and H_2O added are as follows: 1.1µl of pYLR110P+luc template and 9.9µl of water; 1.4µl of pYMR251AP+luc template and 9.6µl of water; 2.0-6.0µl of pYMR107P+luc template and 9.0-5.0µl of water; and 0.5-1.5µl of pZEO1P+luc template and 10.5-9.5µl of water.

The thermocycling protocol is described in the ABI protocol, the PCR products were ethanol precipitated by adding 3M NaOAc and absolute Ethanol, standing at room temperature for 15 minutes, centrifuging for 20 minutes and washing with 250µl of 70% ethanol. The precipitated DNA was resuspended in 3µl of loading dye and 2µl of each suspension was analyzed on an PE-ABI 377 automated sequencer.

The following promoter constructs pYLR110P+luc and pYMR251AP+luc were each sequenced using four primers:

YEp13 F2 : CCTCAATTGGATTAGTCTCA - SEQ ID NO:13- aligns to the YEp13 backbone, 290bp 5= of the Hind III site.

Luc R1 : CACCTCGATATGTGCATCTG - SEQ ID NO:14- aligns to the Luc ORF, 150bp 3= of the NdeI site.

Forward PCR primer: forward primer used to PCR clone promoter, *i.e.*, SEQ ID NO:5 and SEQ ID NO: 7.

Reverse PCR primer: reverse primer used to PCR clone promoter , *i.e.*, SEQ ID NO:6 and SEQ ID NO:8.

The remaining promoter constructs (pYMR107P+luc and pZEO1P+luc) were each sequenced using primers Yep13 F2 and Luc R1. Combining the data from all primers completely sequenced the promoter regions and spanned the cloning sites of the original vector.

Deviations from published genomic sequences

All sequences differ by a few base pairs around the ATG, this results from the creation of an NdeI site at the 3= end of the promoter. In addition, the following further alterations from published sequences were identified.

pYLR110P+luc: A substitution of a C for a T had taken place at a base pair 361 of the sequence.

pYMR107P+luc: In the initial construct (for which luciferase reporter data is described), a cloning artifact led to the junction between the promoter region and the LUC ORF in pYMR107W+luc to have the sequence: CATATATG (where ATG is the luciferase translational start site). This sequence was modified by site directed mutagenesis to create the sequence CATATG , which generates a novel NdeI site at the promoter/luciferase junction. Subsequent luciferase expression analysis confirmed that expression from the NdeI site modified pYMR107P+luc construct did not differ significantly from the original construct, therefore the sequence of the corrected CATATG construct is included herein.

Other Modifications

pYMR107P+luc: Cloning artifacts created an additional HindIII site and linker to the 5= (*i.e.* outside) of the pYMR107P+luc and promoters:

Instead of:

hindIII ***NotI*** **promoter 5=**
AAGCTT-CGCGGCCGCG-NNNNNNN SEQ ID NO:17

The sequence is:

hindIII **hindIII** ***NotI*** **promoter 5=**
AAGCTT-AGCT-AAGCTT-CGCGGCCGCG-NNNNNNN SEQ ID NO:18.

EXAMPLE 4

Luciferase assays of promoter activity

Transformation of *S. cerevisiae* with promoter constructs.

S. cerevisiae strain 11C was transformed with five promoter constructs. This strain carries six metabolic markers, Ade, Trp, Ura, Lys, Leu and His. It has the genotype: *ade2*-161, *trp1*-D63, *ura3*-52, *lys2*-801, *leu2*D1 &/or *leu2*-3 &/or *leu2*-112, *his*D200 &/or *his*D200. 11C was generated by crossing the strains YPH500 (Mat a *ura3*-52 *lys2*-801 *ade2*-161 *trp1*-D63 *his*D200 *leu2*D1) and AH22 (MATa *leu2*-3 *leu2*-112 *his4*-519 *can1*.

11C cells were streaked from a glycerol stock onto a YPD plate and grown at 30°C for two days. The cells were transformed with the five plasmids, pYLR110P+luc, pYMR251AP+luc, pYMR107P+luc, & pZEO1P+luc and pPRB1P+luc to act as a control. The transformations were carried out using the Quick and Easy method (Gietz, R.D. and R.A. Woods, 1994, *Molecular Genetics of Yeast: Practical Approaches* pp. 121-134. 10ml of plasmid was added to the transformation mix in each case. The whole transformation mixes were plated out onto -Leu plates and incubated at 30°C for three days. Three individual colonies from each transformation plate were picked and used to inoculate 10ml YPD cultures. The 10ml cultures were incubated in an orbital shaker set to 200rpm and 30°C. Cells were harvested from the cultures at two points. First, at a point at which the OD of the culture was close to 1.0, at which time a 4ml sample was taken. Second, a 3ml sample was taken after an incubation time of 45 hours. The ODs and incubation time of each sample is shown in Table 5. For all harvested samples, the cells were immediately spun down at 3000rpm and 4°C, washed in 5ml of dH_2O, repelleted and frozen at -20°C.

Table 5

Plasmid	Clone number	OD at time of harvesting first 4ml sample	Incubation time at harvesting of first sample (hours)	OD at time of harvesting second 3ml sample
PPRB1P +luc	7	0.98	24.5	4.80
	8	0.68	28	5.56
	9	1.15	28	5.66
PYLR110P +luc	8	1.12	28	5.50
	9	0.46	28	4.38
	10	1.16	24.5	5.51
PYMR251AP +luc	8	1.20	24.5	4.99
	9	1.05	27	4.71
	10	1.15	27	5.18
PYMR107P +luc	1	1.06	27	5.47
	2	0.49	28.5	4.54
	3	0.97	25.5	5.58
PZEO1P +luc	1	1.02	28.5	4.84
	2	0.62	28.5	4.97
	3	0.42	28.5	4.31

Analysis of luciferase activity

All of the samples were analyzed for luciferase activity, using the LucLite Luciferase Reporter Gene Assay Kit (Packard, cat.no 6016911). The cells were prepared by resuspending in PBS and diluting to a final concentration of 6 x 10^6 cells/ml. 100ml of each cell suspension was pipetted into wells in duplicate on two 96 well plates, so that each well contained 6 x 10^5 cells. The plates were incubated at 30°C for 10 minutes. 100ml of a 1 in 2 dilution of reconstituted substrate was added to each well, and the plate was further incubated at room temperature for 10 minutes. The luminescence was then

measured using the Packard TopCount. The luminescence readings obtained after 0.03min are shown below in counts per second (CPS) in Table 6.

Table 6.

Plasmid	Clone number	First sample				Second sample			
		Readings (CPS)		Average	Average	Readings (CPS)		Average	Average
PPRB1P +luc	7	35890	35690	35790	34898	20322	20975	20648	19867
	8	25498	25276	25387	24495	52997	51778	52388	51607
	9	24137	27797	25967	25075	49192	46971	48081	47300
PYLR110P +luc	8	52354	53618	52986	52094	41789	38904	40346	39565
	9	105299	99776	102537	101645	85562	84468	85015	84234
	10	107531	109226	108379	107486	22507	22436	22471	21690
PYMR251AP +luc	8	71993	69797	70895	70003	40869	40202	40536	39755
	9	98853	98389	98621	97729	51159	49828	50493	49712
	10	83210	87546	85378	84485	70091	74576	72334	71553
PYMR107P +luc	1	9046	8650	8848	6790	29413	28505	28959	28124
	2	3996	4009	4002	1945	24391	23915	24153	23318
	3	3018	3236	3127	1069	23866	23408	23637	22802
PZEO1P +luc	1	64137	63162	63649	61592	47469	45769	46619	45784
	2	19579	18329	18954	16897	44910	42982	43946	43111
	3	87572	90317	88944	86887	142414	142262	142338	141503

The results are summarized in Table 7.

Table 7.

Promoter	mRNA levels	Luciferase Expression Glucose	Luciferase Expression Ethanol
PRB1	Ethanol Induced	1.00	1.00
YLR110C	Highly Ethanol and Glucose Induced	3.03	1.22
YMR251WA	Highly Ethanol and Glucose Induced	2.92	1.35
YMR107W	Ethanol Induced	0.21	0.95
ZEO1	Very Highly Ethanol	3.62	2.89

	and Glucose Induced		

Three promoters give higher levels of expression than PRB1 at both ODs, these are: YLR110C, YMR251WA, and ZEO1. The promoter showing the greatest fold induction is YMR107W.

Creating vectors with promoters but without the luciferase gene

Based on the analysis of luciferase expression four further promoter constructs have been made. The lack the luciferase gene and can be used to clone nucleic acid molecules encoding polypeptides of interest downstream of the promoters such that they drive expression of the nucleic molecules of interest. The sequences of these four plasmids are named: G1: pYLR110P (SEQ ID NO:23) (map at Figure 9); G2: pYMR251AP (SEQ ID NO:24) (map at Figure 10); G3 pYMR107P (SEQ ID NO:25) (map at Figure 11); and G4: pZEO1P (SEQ ID NO:26) (map at Figure 12). These were constructed by digesting pPRB1P (SEQ ID NO:27) with HindIII and NdeI to obtain the vector. The promoter+luc construct was digested with HindIII and NdeI to obtain the promoter fragment. The vector and promoter DNA was purified from LMP agarose using PCRpreps. The vector and promoter was ligated and used to transform *E. coli*. Correct recombinants were screened for.

EXAMPLE 5

Isolation of Active Promoter Fragments

Operative fragments of the YLR110C, YMR251WA, YMR107W and ZEO1 promoters can be generated using restriction endonucleases, 5' or 3' deletion mutagenesis, PCR, site specific deletion, or a combination thereof. For example, purified pYLR110P+luc,

pYMR251AP+luc, pYMR107P+luc or pZEO1P+luc plasmids, as generated in Example 3, can be subjected to restriction endonucleases to generate fragments of the YLR110C, YMR251WA, YMR107W or ZEO1 promoters. Restriction endonuclease sites, preferably unique restriction endonuclease sites, within the promoter sequences shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4 can be identified that generate fragments of the promoter upon restriction endonuclease digestion. Such fragments are preferably, 17, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650 or 700 nucleotides in length.

The fragments generated by restriction endonuclease digestion of the promoters shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4 can be separated by agarose gel electrophoresis. The agarose gel band corresponding to the desired promoter fragment can be cut out of the agarose gel. The fragment can be isolated and purified from the agarose gel by, for example, electroelution or kits such as QIAquick™ gel extraction kit or QIAEX7 II Gel Extraction System (Qiagen Cat. No. 28704 and 20021).

The purified promoter fragment can be ligated into the isolated and purified HindIII, NdeI, double-digested pPRBP1+luc backbone such that the promoter fragment is operably linked to a luciferase gene and transformed into *E. coli*, as described in Example 3. The new expression vector comprising a fragment of YLR110C, YMR251WA, YMR107W, or ZEO1 promoter region can be isolated and purified from *E. coli*, sequenced, and transformed into yeast as described in Example 3.

To analyze promoter activity, luciferase assays as described in Example 4, can be conducted using *S. cerevisiae* cultures that have been transformed with the expression vector comprising a fragment of the YLR110C, YMR251WA, YMR107W, or ZEO1 promoter operably linked to a luciferase gene and *S. cerevisiae* cultures that have been transformed with pPRB1P+luc. The *S. cerevisiae* cultures are grown in medium containing a non-fermentable carbon source, such as ethanol, or a fermentable carbon source, such as glucose, or both. Cells are obtained from the cultures and analyzed for luciferase activity as described in Example 4.

A promoter fragment is operative if it expresses at least 75% of the luciferase activity as the PRB1 promoter. Preferably, an operative promoter fragment expresses at least 100%, 200%, 300%, 400%, or more of the luciferase activity as the PRB1 promoter.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 Polynucleotide sequence of promoter YLR110C

SEQ ID NO:2 Polynucleotide sequence of promoter YMR251WA

SEQ ID NO:3 Polynucleotide sequence of promoter YMR107W

SEQ ID NO:4 Polynucleotide sequence of promoter ZEO1

SEQ ID NO:5 Forward PCR primer for YLR110C

SEQ ID NO:6 Reverse PCR primer for YLR110C

SEQ ID NO:7 Forward PCR primer for YMR251WA

SEQ ID NO:8 Reverse PCR primer for YMR251WA

SEQ ID NO: 9 Forward PCR primer for YMR107W

SEQ ID NO:10 Reverse PCR primer for YMR107W

SEQ ID NO:11 Forward PCR primer for ZEO1

SEQ ID NO:12 Reverse PCR primer for ZEO1

SEQ ID NO:13: Yep13 Forward PCR primer

SEQ ID NO:14: Luc RI Forward PCR primer

SEQ ID NO:15 Primer used in cDNA sequencing

SEQ ID NO:16 Control oligonucleotide used in GeneChip Microarray assay

SEQ ID NO:17 Original pYMR107P+luc sequence

SEQ ID NO:18 Modified pYMR107P+luc sequence

SEQ ID NO:19 Nucleotide sequence of pYLR110P+luc

SEQ ID NO:20 Nucleotide sequence of pYMR251AP+luc

SEQ ID NO:21 Nucleotide sequence of pYMR107P+luc

SEQ ID NO:22 Nucleotide sequence of pZEO1P+luc

SEQ ID NO:23 Nucleotide sequence of pYLR110P

SEQ ID NO:24 Nucleotide sequence of pYMR251AP

SEQ ID NO:25 Nucleotide sequence of pYMR107P

SEQ ID NO:26 Nucleotide sequence of pZEO1P

SEQ ID NO:27 Nucleotide sequence of pPRB1P

SEQ ID NO:28 Nucleotide sequence of pPRB1P+luc

SEQ ID NO:29 YLR110C promoter region

SEQ ID NO:30 YMR251WA promoter region

SEQ ID NO:31 YMR107W promoter region

SEQ ID NO:32 ZEO1 promoter region

CLAIMS

1. A yeast promoter which comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, wherein the promoter is operative to express a nucleic acid molecule encoding a polypeptide when operably linked to said nucleic acid molecule.

2. The yeast promoter of claim 1, wherein the promoter comprises at least 50 contiguous nucleotides of an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

3. The yeast promoter of claim 1, wherein the promoter comprises at least 100 contiguous nucleotides of an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

4. The yeast promoter of claim 1, wherein the promoter comprises at least 200 contiguous nucleotides of an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

5. The yeast promoter of claim 1, wherein the promoter comprises at least 300 contiguous nucleotides of an isolated and purified polynucleotide selected

from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

6. The yeast promoter of claim 1, wherein the promoter comprises at least 400 contiguous nucleotides of an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

7. A yeast promoter which comprises an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

8. A yeast promoter fragment which comprises at least 17 contiguous nucleotides of a polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, wherein the fragment has promoter activity as determined by the steps of:

(a) cloning the fragment into a yeast expression vector, wherein the fragment is operably linked to a reporter gene;

(b) transforming yeast cells with the yeast expression vector;

(c) growing the yeast cells in yeast cell culture under conditions favorable for expression of the reporter gene; and

(d) assaying the yeast culture for a reporter protein expressed by the reporter gene;

wherein expression of the reporter gene indicates the fragment has promoter activity.

9. A yeast expression vector comprising the yeast promoter of claim 1.

10. The yeast expression vector of claim 9 wherein the yeast expression vector is selected from the group consisting of pYLR110P+luc, pYMR251AP+luc, pYMR107P+luc, pZEO1P+luc, pYLR110P, pYMR251AP, pYMR107P, and pZEO1P.

11. The yeast expression vector of claim 9 wherein activity of the promoter is controlled by varying the level of a non-fermentable carbon source in a medium of yeast cells in culture, wherein the yeast cells are transformed with said yeast expression vector.

12. The yeast expression vector of claim 11 wherein the non-fermentable carbon source is ethanol.

13. A yeast expression vector comprising a yeast promoter which comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:4, wherein the promoter is operative to express a nucleic acid molecule encoding a polypeptide when operably linked to said nucleic acid molecule, wherein promoter activity is controlled by varying the level of a fermentable carbon source in a medium of yeast cells in culture, wherein the yeast cells are transformed with said yeast expression vector.

14. The yeast expression vector of claim 13 wherein the fermentable carbon source is glucose.

15. A yeast expression vector comprising a yeast promoter which comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:4, wherein the promoter is operative to express a nucleic acid molecule when operably linked to said nucleic acid molecule, wherein promoter activity is controlled by varying the level of a fermentable carbon source and a non-fermentable carbon source in a medium of yeast cells in culture, wherein the yeast cells are transformed with said yeast expression vector.

16. The yeast expression vector of claim 15 wherein the fermentable carbon source is glucose.

17. The yeast expression vector of claim 15 wherein the non-fermentable carbon source is ethanol.

18. A yeast cell transformed with the yeast expression vector of claim 9.

19. A yeast cell transformed with the yeast expression vector of claim 10.

20. A method for producing a polypeptide comprising the steps of:

(a) constructing a yeast expression vector wherein a nucleic acid encoding the polypeptide is controlled by the yeast promoter of claim 1;

(b) transforming a culture of yeast cells with the yeast expression vector;

(c) maintaining the yeast cells in culture so that the polypeptide is expressed; and

(d) recovering the polypeptide.

21. A method for producing a polypeptide comprising the steps of:

(a) cloning a nucleic acid molecule encoding the polypeptide into an expression vector selected from the group consisting of pYLR110P+luc, pYMR251AP+luc, pYMR107P+luc, pZEO1P+luc, pYLR110P, pYMR251AP, pYMR107P, and pZEO1P, wherein the nucleic acid molecule is operably linked to a promoter of the expression vector;

(b) transforming a culture of yeast cells with the yeast expression vector;

(c) maintaining the yeast cells in culture so that the polypeptide is expressed; and

(d) recovering the polypeptide.

22. A method for producing a polypeptide comprising the steps of:

(a) constructing a yeast expression vector wherein a nucleic acid molecule encoding the polypeptide is controlled by a yeast promoter which comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:4;

(b) transforming a culture of yeast cells with the yeast expression vector;

(c) maintaining the yeast cells in culture medium and controlling the expression of the nucleic acid molecule encoding the polypeptide by varying the level of a fermentable carbon source in the culture medium; and

(d) recovering the polypeptide.

23. The method of claim 22 wherein the fermentable carbon source is glucose.

24. A method for producing a polypeptide comprising the steps of:

(a) constructing a yeast expression vector wherein a nucleic acid molecule encoding the polypeptide is controlled by the yeast promoter of claim 1;

(b) transforming a culture of yeast cells with the yeast expression vector;

(c) maintaining the yeast cells in culture medium and controlling the expression of the nucleic acid molecule encoding the polypeptide by varying the level of a non-fermentable carbon source in the culture medium; and

(d) recovering the polypeptide.

25. The method of claim 24 wherein the non-fermentable carbon source is ethanol.

26. A method for producing a polypeptide comprising the steps of:

(a) constructing a yeast expression vector wherein a nucleic acid molecule encoding the polypeptide is controlled by a yeast promoter which comprises at least 17 contiguous nucleotides of an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:4;

(b) transforming a culture of yeast cells with the yeast expression vector;

(c) maintaining the yeast cells in culture medium and controlling the expression of the nucleic acid molecule encoding the polypeptide by varying the level of a fermentable carbon source and a non-fermentable carbon source in the culture medium; and

(d) recovering the polypeptide.

27. The method of claim 26 wherein the fermentable carbon source is glucose.

28. The method of claim 26 wherein the non-fermentable carbon source is ethanol.

29. A method of identifying a promoter fragment, wherein the fragment has promoter activity comprising the steps of:

(a) generating a fragment comprising at least 17 contiguous nucleotides of an isolated and purified polynucleotide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4;

(b) cloning the fragment into a yeast expression vector, wherein the fragment is operably linked to a reporter gene;

(c) transforming yeast cells with the yeast expression vector;

(d) growing the yeast cells in yeast cell culture under conditions favorable for expression of the reporter gene; and

(e) assaying the yeast culture for a reporter protein expressed by the reporter gene;

wherein expression of the reporter gene indicates the fragment has promoter activity.

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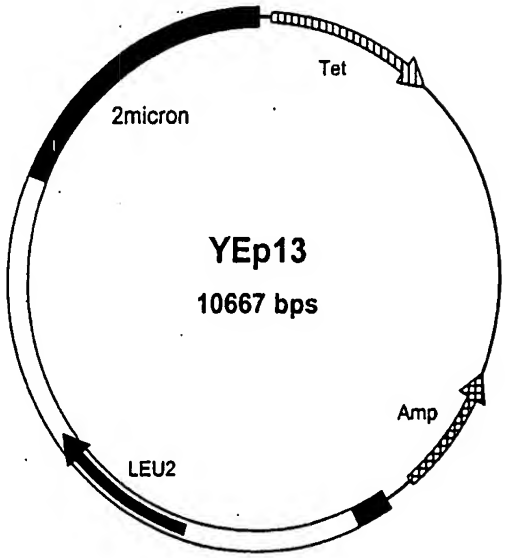


FIGURE 1

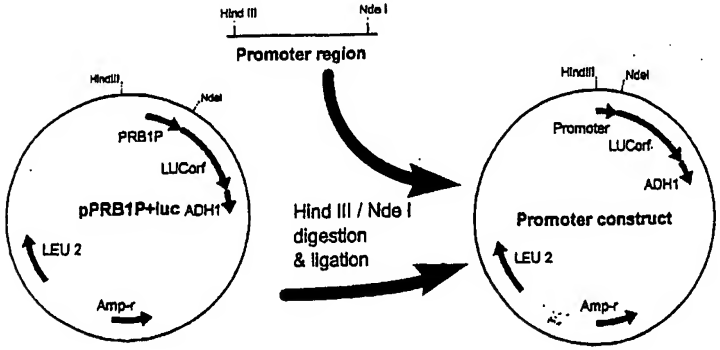


FIGURE 2

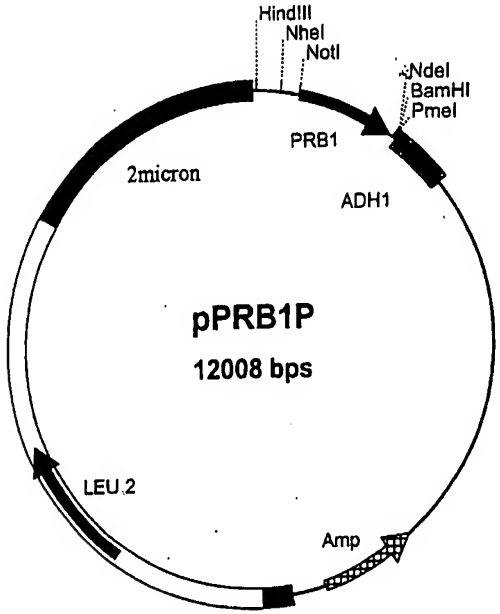


FIGURE 3

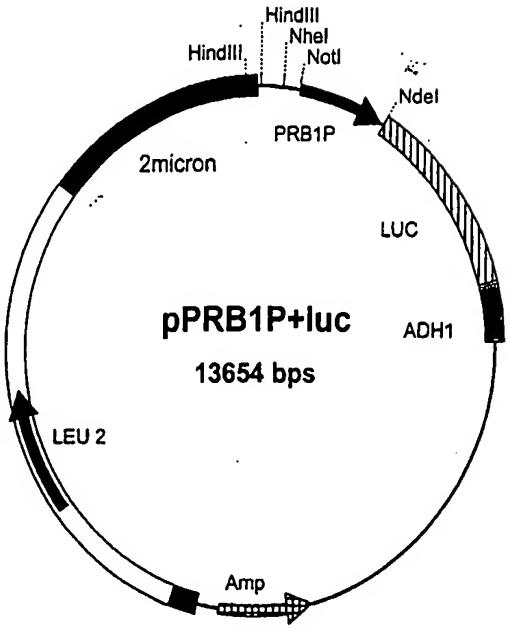
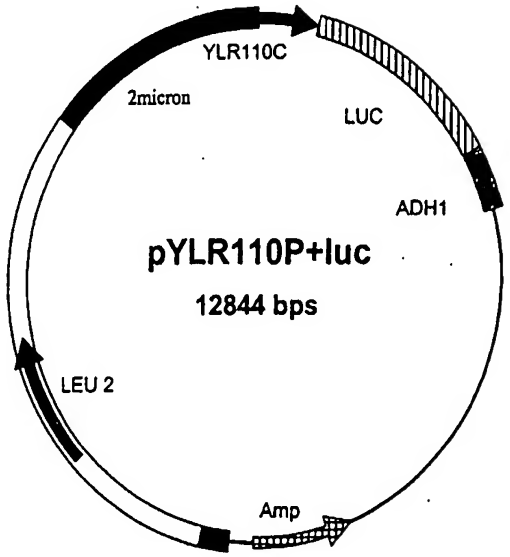


FIGURE 4

WO 01/38549 PCT/SE00/02277



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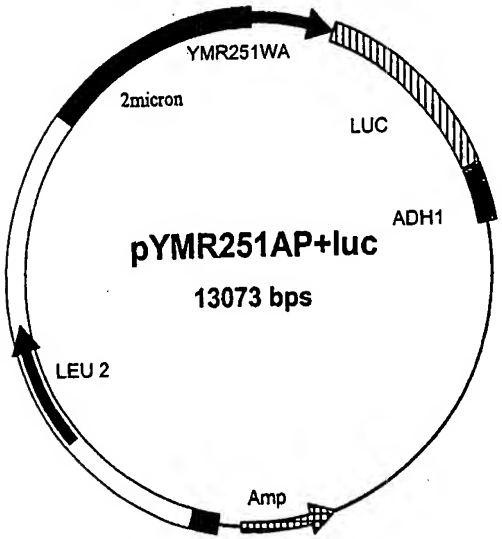

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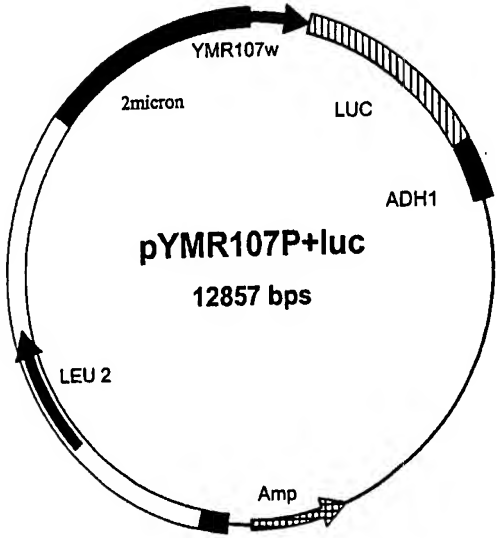

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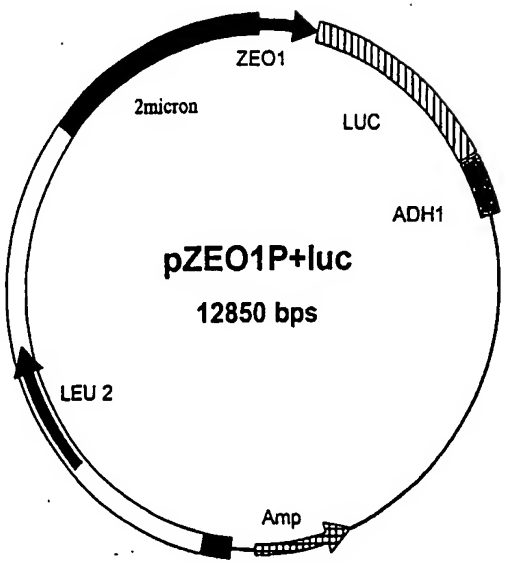

FIGURE 8

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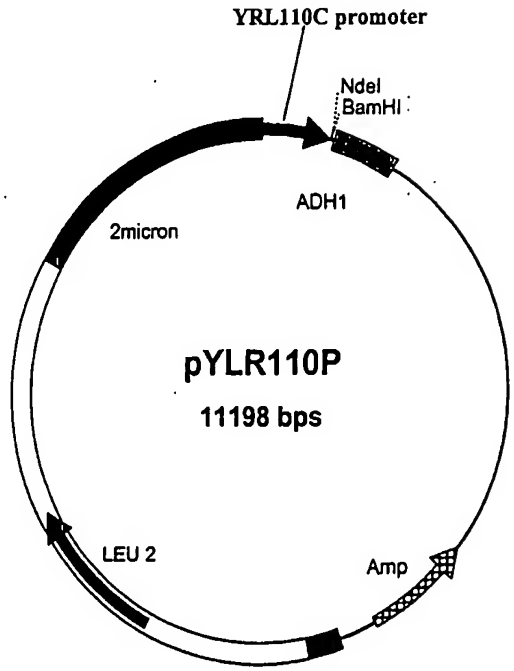

FIGURE 9

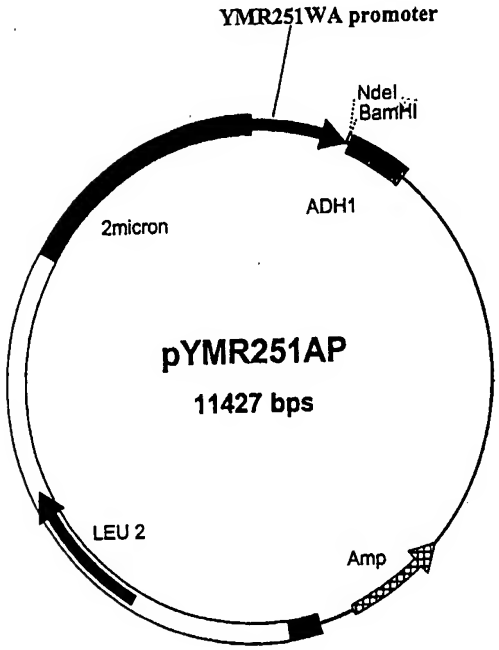

FIGURE 10

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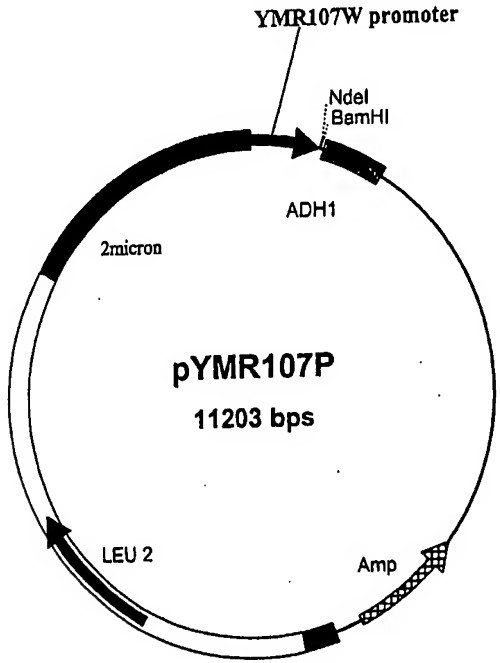

FIGURE 11

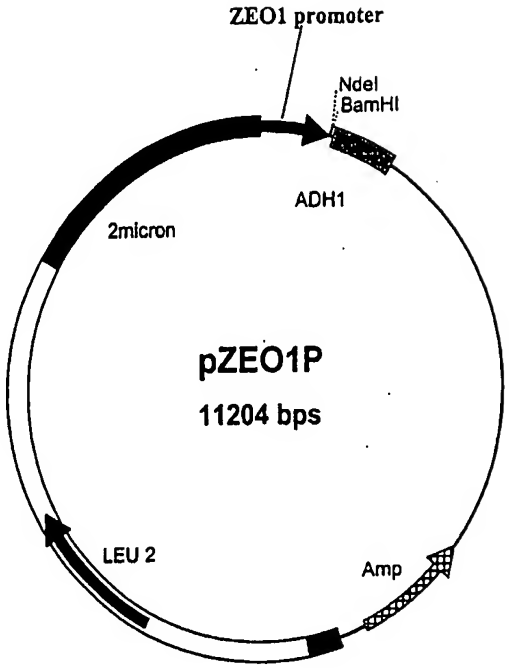

FIGURE 12

Figure 13 YLR110C promoter region (SEQ ID NO:29)
Sequence shown: Chr XII 370650 to 370051 (reverse orientation)

```
      1  AGAACCAAAT GGGAAAATCG GAATGGGTCC AGAACTGCTT TGAGTGCTGG
         TCTTGGTTTA CCCTTTTAGC CTTACCCAGG TCTTGACGAA ACTCACGACC
ATGCAAGCTTCGCGGCCGC              YLR-F
     51  CTATTGGCGT CTGATTTCCG TTTTGGGAAT CCTTTGCCGC GCGCCCCTCT
         GATAACCGCA GACTAAAGGC AAAACCCTTA GGAAACGGCG CGCGGGGAGA

    101  CAAAACTCCG CACAAGTCCC AGAAAGCGGG AAAGAAATAA AACGCCACCA
         GTTTTGAGGC GTGTTCAGGG TCTTTCGCCC TTTCTTTATT TTGCGGTGGT

    151  AAAAAAAAAA AATAAAAGCC AATCCTCGAA GCGTGGGTGG TAGGCCCTGG
         TTTTTTTTTT TTATTTTCGG TTAGGAGCTT CGCACCCACC ATCCGGGACC

    201  ATTATCCCGT ACAAGTATTT CTCAGGAGTA AAAAAACCGT TTGTTTTGGA
         TAATAGGGCA TGTTCATAAA GAGTCCTCAT TTTTTTGGCA AACAAAACCT

    251  ATTCCCCATT TCGCGGCCAC CTACGCCGCT ATCTTTGCAA CAACTATCTG
         TAAGGGGTAA AGCGCCGGTG GATGCGGCGA TAGAAACGTT GTTGATAGAC

    301  CGATAACTCA GCAAATTTTG CATATTCGTG TTGCAGTATT GCGATAATGG
         GCTATTGAGT CGTTTAAAAC GTATAAGCAC AACGTCATAA CGCTATTACC

    351  GAGTCTTACT TCCAACATAA CGGCAGAAAG AAATGTGAGA AAATTTTGCA
         CTCAGAATGA AGGTTGTATT GCCGTCTTTC TTTACACTCT TTTAAAACGT

    401  TCCTTTGCCT CCGTTCAAGT ATATAAAGTC GGCATGCTTG ATAATCTTTC
         AGGAAACGGA GGCAAGTTCA TATATTTCAG CCGTACGAAC TATTAGAAAG

    451  TTTCCATCCT ACATTGTTCT AATTATTCTT ATTCTCCTTT ATTCTTTCCT
         AAAGGTAGGA TGTAACAAGA TTAATAAGAA TAAGAGGAAA TAAGAAAGGA

    501  AACATACCAA GAAATTAATC TTCTGTCATT CGCTTAAACA CTATATCAAT
         TTGTATGGTT CTTTAATTAG AAGACAGTAA GCGAATTTGT GATATACTTA
                                                  YLR-R      GT
    551  AATGCAATTT TCTACTGTCG CTTCTATCGC CGCTGTCGCC GCTGTCGCTT
         TTACGTTAAA AGATGACAGC GAAGATAGCG GCGACAGCGG CGACAGCGAA
         A   CCGGACC
```

YLR111W ORF = Underline

YLR110C ORF = Bold

YLR-F = SEQ ID NO:5

YLR-R = SEQ ID NO:6

Figure 14 YMR251WA promoter region (SEQ ID NO:30)

Sequence shown: CHR XIII 773951 TO 774800

```
1    GCCACGGGTC AACCCGATTG GGATCACCCC ACTGGGGCCC AAGCCTGATA
     CGGTGCCCAG TTGGGCTAAC CCTAGTGGGG TGACCCCGGG TTCGGACTAT
               AGCTAAGCTTCGCGGCCGC                YMR-F

51   TCCGACCTCC ATGAAATTTT TTTTTTTCTT TCGATTAGCA CGCACACACA
     AGGCTGGAGG TACTTTAAAA AAAAAAAGAA AGCTAATCGT GCGTGTGTGT

101  TCACATAGAC TGCGTCATAA AAATACACTA CGGAAAAACC ATAAAGAGCA
     AGTGTATCTG ACGCAGTATT TTTATGTGAT GCCTTTTTGG TATTTCTCGT

151  AAGCGATACC TACTTGGAAG GAAAAGGAGC ACGCTTGTAA GGGGGATGGG
     TTCGCTATGG ATGAACCTTC CTTTTCCTCG TGCGAACATT CCCCCTACCC

201  GGCTAAGAAG TCATTCACTT TCTTTTCCCT TCGCGGTCCG GACCCGGGAC
     CCGATTCTTC AGTAAGTGAA AGAAAAGGGA AGCGCCAGGC CTGGGCCCTG

251  CCCTCCTCTC CCCGCACGAT TTCTTCCTTT CATATCTTCC TTTTATTCCT
     GGGAGGAGAG GGGCGTGCTA AAGAAGGAAA GTATAGAAGG AAAATAAGGA

301  ATCCCGTTGA AGCAACCGCA CTATGACTAA ATGGTGCTGG ACATCTCCAT
     TAGGGCAACT TCGTTGGCGT GATACTGATT TACCACGACC TGTAGAGGTA

351  GGCTGTGACT TGTGTGTATC TCACAGTGGT AACGGCACCG TGGCTCGGAA
     CCGACACTGA ACACACATAG AGTGTCACCA TTGCCGTGGC ACCGAGCCTT

401  ACGGTTCCTT CGTGACAATT CTAGAACAGG GGCTACAGTC TCGATAATAG
     TGCCAAGGAA GCACTGTTAA GATCTTGTCC CCGATGTCAG AGCTATTATC

451  AATAATAAGC GCATTTTTGC TAGCGCCGCC GCGGCGCCCG TTTCCCAATA
     TTATTATTCG CGTAAAAACG ATCGCGGCGG CGCCGCGGGC AAAGGGTTAT

501  GGGAGGCGCA GTTTATCGGC GGAGCTCTAC TTCTTCCTAT TTGGGTAAGC
     CCCTCCGCGT CAAATAGCCG CCTCGAGATG AAGAAGGATA AACCCATTCG

551  CCCTTTCTGT TTTCGGCCAG TGGTTGCTGC AGGCTGCGCC GGAGAACATA
     GGGAAAGACA AAAGCCGGTC ACCAACGACG TCCGACGCGG CCTCTTGTAT

601  GTGATAAGGG ATGTAACTTT CGATGAGAGA ATTAGCAAGC GGAAAAAAAC
     CACTATTCCC TACATTGAAA GCTACTCTCT TAATCGTTCG CCTTTTTTTG

651  TATGGCTAGC TGGGAGTTGT TTTTCAATCA TATAAAAGGG AGAAATTGTT
     ATACCGATCG ACCCTCAACA AAAAGTTAGT ATATTTTCCC TCTTTAACAA

701  GCTCACTATG TGACAGTTTC TGGGACGTCT TAACTTTTAT TGCAGAGGAC
     CGAGTGATAC ACTGTCAAAG ACCCTGCAGA ATTGAAAATA ACGTCTCCTG

751  TATCAAATCA TACAGATATT GTCAAAAAAA AAAAAGACTA ATAATAAAAA
     ATAGTTTAGT ATGTCTATAA CAGTTTTTTT TTTTTCTGAT TATTATTTTT
                                          YMR-R      G A

801  ATGAAGTTAT CTCAAGTTGT TGTTTCCGCC GTCGCCTTCA CTGGTTTAGT
     TACTTCAATA GAGTTCAACA ACAAAGGCGG CAGCGGAAGT GACCAAATCA
           C
```

YMR251W ORF = Underline
YMR251WA ORF = Bold
YMR-F = SEQ ID NO:7
YMR-R = SEQ ID NO:8

Figure 15 YMR107W PROMOTER REGION (SEQ ID NO:31)

Sequence shown: CHR XIII 482463 TO 483063

```
1    AAAGAATCCA TCACTATTTG AAAAAAAGTC ATCTGGCACG TTTAATTATC
YMR107-F
AGCTAAGCTTCGCGGCCGC
51   AGAGCAGAAA TGATGAAGGG TGTTAGCGCC GTCCACTGAT GTGCCTGGTA

101  GTCATGATTT ACGTATAACT AACACATCAT GAGGACGGCG GCGTCACCCC

151  AACGCAAAAG AGTGACTTCC CTGCGCTTTG CCAAAACCCC ATACATCGCC

201  ATCTGGCTCC TGGCAGGGCG GTTGATGGAC ATCAGCCGCC TCCCTTAATT

251  GCTAAAGCCT CCACAAGGCA CAATTAAGCA ATATTTCGGG AAAGTACACC

301  AGTCAGTTTG CGCTTTTATG ACTGGGTTCT AAGGTACTAG ATGTGAAGTA

351  GTGGTGACAG AATCAGGGAG ATAAGAGGGA GCAGGGTGGG GTAATGATGT

401  GCGATAACAA TCTTGCTTGG CTAATCACCC CCATATCTTG TAGTGAGTAT

451  ATAAATAGGA GCCTCCCTTC CTATTGCAAC TCCATAAAAT TTTTTTTTGT
                                                MODIFICATION AT
501  AGCCACTTCT GTAACAAGAT AAATAAAACC AACTAATCGA GATATCAAAT
                                      GATTAGCT CTATAGTGTA

551  ATGGGTAGTT TTTGGGACGC ATTCGCAGTA TACGACAAGA AAAAGCACGC
     TACCCTACCTA YMR107-R
```

YMR107W ORF = Bold
YMR107-F = SEQ ID NO:9
YMR107-R = SEQ ID NO:10

Figure 16 ZEO1 PROMOTER REGION (SEQ ID NO:32)

Sequence shown: CHR XV 109746 TO 110346

```
                1 TTCAGGAGTC TCTCGCGTTA GAGCAGTACG TGGCGCAGCT AAACTCGCCG
ZEO1-F
AGCTAAGCTTCGCGGCCGC
               51 GGAGGTCTGCTTCACGAGCG CGGTGTGCGC CTAGTATTGC CCCGACGGTC

              101 CGGGTGCCTA TCCCTAGATT TCGTCGTGCC CCGACCCAAA TAGTTAAACG

              151 TGTGGTTTAT GGGTGCACCA GGGCTTTATC GTGTTTTATA TCGATGGCGA

              201 TTTGTGCCTC CAGTGTATTT TTGTATATCC AATTAAGGTT TCTTACCTAA

              251 TTTTATTTTT ATCATCTTTA GTTAATGCTG GTTTGCTCTG TTTCTGCTGC

              301 TTTCTGTGCG GTTCTCCTCT TCTCTTGTTT CTTCGTGTTG TCCCCCATCG

              351 CCGATGGGCT TATATGGCGT ATATATATAG AGCGAGTTTT TACGTCGAAG

              401 ATCATCTCAG TTTGCTTGAT AGCCTTTCTA CTTTATTACT TTCGTTTTTA

              451 ACCTCATTAT ACTTTAGTTT TCTTTGATCG GTTTTTTTCT CTGTATACTT

              501 AAAAGTTCAA ATCAAAGAAA CATACAAAAC TACGTTTATA TCAATTAATA
                                                  GCAAATAT AGTTAATGTA

              551 ATGTCTGAAA TTCAAAACAA AGCTGAAACT GCCGCCCAAG ATGTCCAACA
                  TACGCTAGCAT ZEO1-R
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ZEO1 (YOL109W) ORF = Bold
ZEO1-F = SEQ ID NO:11
ZEO1-R = SEQ ID NO:12

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agccgcctcc cttaattgct aaagcctcca caaggcacaa ttaagcaata tttcgggaaa 240

gtacaccagt cagtttgcgc ttttatgact gggttctaag gtactagatg tgaagtagtg 300

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tcaattaata atgtctgaaa ttcaaaacaa agctgaaact gccgcccaag atgtccaaca 600

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B. FIELDS SEARCHED

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IPC7: C12N

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SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOHNSTON, M. et al. "The nucleotide sequence of Saccharomyces cervisiae chromosome XII". NATURE, 29 May 1997, Vol 387, pages 87-90, see especially page 88, left-hand column, paragraph 3 --	1-29
X	VANDENBOL, Micheline et al. "Sequence Analysis of a 44 kb DNA Fragment of Yest Chromosome XV Including the Ty1-H3 Retrotransposon, the suf1(+) Frameshift Suppressor Gene for tRNA-Gly, the Yest Transfer RNA-Thr-1a and a Delta Element". YEAST, 1995, Vol. 11, pages 1069-1075, see entire document --	1-29

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 March 2001	08-03-2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Henrik Nilsson /OGU Telephone No. +46 8 782 25 00

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VERHASSELT, Peter et al. "Sequence Analysis of a 37.6kbp Cosmid Clone from the Right Arm of Saccharomyces cerevisiae Chromosome XII, Carrying YAP3, HOG1, SNR6, tRNA-Arg3 and 23 New Open Reading Frames, Among Which Several Homologies to Proteins Involved in Cell Division Control and to Mammalian Growth Factors and other Animal Proteins are Found" YEAST, 1997, Vol. 13, pages 241-250, see especially page 249, right hand-column, paragraph 3 --	1-29
X	EMBL/GenBank/DDBJ Databases, HUNT, S. et al: "S. cerevisiae chromosome XIII cosmid 9718" 1995-05-21, Accession No. Z49702 --	1-29
X	EMBL/GenBank/DDBJ Databases, HUNT, S. et al: "S. cerevisiae chromosome XIII cosmid 9920" 1995-03-12, Accession No. Z48639 -- --------	1-29

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【発明者】
【住所又は居所】　愛知県豊田市トヨタ町１番地　トヨタ自動車株式会社内
【氏名】　安藤　大吾

【発明者】
【住所又は居所】　愛知県豊田市トヨタ町１番地　トヨタ自動車株式会社内
【氏名】　井上　敏夫

【発明者】
【住所又は居所】　愛知県豊田市トヨタ町１番地　トヨタ自動車株式会社内
【氏名】　戸祭　衛

【発明者】
【住所又は居所】　愛知県豊田市トヨタ町１番地　トヨタ自動車株式会社内
【氏名】　長谷川　景子

【発明者】
【住所又は居所】　愛知県豊田市トヨタ町１番地　トヨタ自動車株式会社内
【氏名】　伏木　俊介

【発明者】
【住所又は居所】　愛知県豊田市トヨタ町１番地　トヨタ自動車株式会社内
【氏名】　安部　司

【特許出願人】
【識別番号】　000003207
【氏名又は名称】　トヨタ自動車株式会社

【代理人】
【識別番号】　110000017
【氏名又は名称】　特許業務法人アイテック国際特許事務所
【代表者】　伊神　広行
【電話番号】　052-218-3226
【連絡先】　担当は田中敏博

【手数料の表示】
【予納台帳番号】　008268
【納付金額】　16,000円

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【物件名】　特許請求の範囲　1
【物件名】　明細書　1
【物件名】　図面　1
【物件名】　要約書　1
【包括委任状番号】　0104390

【書類名】特許請求の範囲
【請求項１】
内燃機関と、
前記内燃機関の吸気バルブの開閉タイミングを変更可能な開閉タイミング変更手段と、
吸気バルブの開閉タイミングが前記内燃機関の圧縮行程における筒内の圧力である筒内圧縮圧力を通常時よりも低減する遅角側の所定タイミングとなる位置で前記開閉タイミング手段変更手段を固定可能な固定手段と、
前記固定手段の状態を検出する検出手段と、
所定のアイドル条件が成立し前記検出手段によって前記固定手段が前記所定タイミングとなる位置での固定を解除した状態であると検出されたときには前記所定タイミングよりも進角した位置で前記内燃機関をアイドル運転する通常時アイドル制御を実行するよう前記内燃機関と前記開閉タイミング変更手段とを制御し、所定のアイドル条件が成立し前記検出手段によって前記固定手段が前記所定タイミングとなる位置での固定を解除できない状態であると検出されたときには前記通常時アイドル制御と異なる固定時制御を実行するよう前記内燃機関を制御する制御手段と、
を備えた内燃機関装置。
【請求項２】
請求項１に記載の内燃機関装置であって、
駆動軸に動力の入出力が可能な電動機と、
前記内燃機関の出力軸と前記駆動軸とに接続され、電力と動力との入出力を伴って該内燃機関からの動力の少なくとも一部を前記駆動軸に出力可能な電力動力入出力手段と、
前記電動機と前記電力動力入出力手段と電力のやり取りが可能な蓄電手段と、
を備えた内燃機関装置。
【請求項３】
前記電力動力入出力手段は、前記駆動軸と前記内燃機関の出力軸と回転可能な回転軸の３軸に接続され該３軸のうちのいずれか２軸に入出力される動力に基づいて残余の軸に動力を入出力する３軸式動力入出力手段と、前記回転軸に動力を入出力可能な発電機と、を備える手段である、請求項２に記載の内燃機関装置。
【請求項４】
前記制御手段は、前記固定時制御を実行するに際して、前記蓄電手段に電力が蓄電されるよう前記内燃機関と前記電動機と前記電力動力入出力手段とを制御する蓄電制御を実行する、請求項２又は３に記載の内燃機関装置。
【請求項５】
前記制御手段は、前記固定時制御を実行するに際して、前記蓄電手段に電力を蓄電させる蓄電制御と前記内燃機関の運転停止とを前記蓄電手段の残容量に基づいて切り替えて実行するよう前記内燃機関と前記電動機と前記電力動力入出力手段とを制御する、請求項２又は３に記載の内燃機関装置。
【請求項６】
前記制御手段は、前記蓄電制御と前記内燃機関の運転停止とを前記蓄電手段の残容量に基づいて切り替えて実行するに際して、前記蓄電手段の残容量が所定値未満であるときには前記蓄電制御を実行するよう前記内燃機関と前記電動機と前記電力動力入出力手段とを制御し、前記蓄電手段の残容量が前記所定値以上であるときには前記内燃機関の運転を停止させる、請求項５に記載の内燃機関装置。
【請求項７】
前記制御手段は、前記固定時制御を実行するに際して、前記内燃機関の運転を停止させる、請求項１～３のいずれかに記載の内燃機関装置。
【請求項８】
前記制御手段は、前記固定時制御を実行するに際して、前記通常時アイドル制御よりも高い回転数で前記内燃機関をアイドル運転するよう前記内燃機関を制御する、請求項１～３のいずれかに記載の内燃機関装置。

【請求項９】
請求項１～８のいずれかに記載の内燃機関装置を搭載し、車軸が前記駆動軸に連結されてなる車両。
【請求項１０】
内燃機関と、前記内燃機関の吸気バルブの開閉タイミングを変更可能な開閉タイミング変更手段と、吸気バルブの開閉タイミングが通常時よりもコンプレッション仕事を低減する遅角側の所定タイミングとなる位置で前記開閉タイミング手段変更手段を固定可能な固定手段とを備えた内燃機関装置の制御方法であって、
所定のアイドル条件が成立し前記固定手段が前記所定タイミングとなる位置での固定を解除した状態であるときには前記所定タイミングよりも進角した位置で前記内燃機関をアイドル運転する通常時アイドル制御を実行するよう前記内燃機関と前記開閉タイミング変更手段とを制御し、所定のアイドル条件が成立し前記固定手段が前記所定タイミングとなる位置での固定を解除できない状態であるときには前記通常時アイドル制御と異なる固定時制御を実行するよう前記内燃機関を制御する、
内燃機関装置の制御方法。

【書類名】明細書
【発明の名称】内燃機関装置、それを搭載した車両及び内燃機関装置の制御方法
【技術分野】
【０００１】
本発明は、内燃機関装置、それを搭載した車両及び内燃機関装置の制御方法に関する。
【背景技術】
【０００２】
従来、内燃機関装置としては、油圧式のアクチュエータを用いて内燃機関の吸気バルブの開閉タイミングを変更するバルブタイミング可変機構と、このバルブタイミング可変機構を最進角位置又は最遅角位置で固定可能なロックピンとを備えたものが知られている（例えば、特許文献１参照）。この装置では、内燃機関の温度が低いときには最進角位置でバルブタイミング可変機構をロックピンにより固定して圧縮圧を高くして内燃機関を始動することにより冷間時の始動性を向上させ、内燃機関の温度が高いときには最遅角位置でバルブタイミング可変機構をロックピンにより固定して圧縮圧を低くして内燃機関を始動することにより始動時の振動を減少させる。そして、内燃機関の始動後は油圧を用いてロックピンによるバルブタイミング可変機構の固定を解除して任意の開閉タイミングで内燃機関の運転を行う。
【特許文献１】特開２０００－３２０３５６号公報
【発明の開示】
【発明が解決しようとする課題】
【０００３】
しかしながら、この特許文献１に記載された内燃機関装置では、バルブタイミング可変機構を駆動する油温が高い場合など油の粘性が低いときなどには、油圧がうまく作用せず、ロックピンが抜けにくくなることがあった。そして、圧縮圧を低減する遅角位置でバルブタイミング可変機構が固定されたまま内燃機関のアイドル運転を実行してしまうと、通常のアイドル運転時に比べ内燃機関から出力される動力が小さくなるなどして、振動が発生してしまうことがあった。
【０００４】
本発明は、このような課題に鑑みなされたものであり、吸気バルブの開閉タイミングを変更可能な内燃機関のアイドル運転において振動の発生を抑制することができる内燃機関装置、それを搭載した車両及び内燃機関装置の制御方法を提供することを目的とする。
【課題を解決するための手段】
【０００５】
本発明は、上述の目的を達成するために以下の手段を採った。
【０００６】
本発明の内燃機関装置は、
内燃機関と、
前記内燃機関の吸気バルブの開閉タイミングを変更可能な開閉タイミング変更手段と、
吸気バルブの開閉タイミングが前記内燃機関の圧縮行程における筒内の圧力である筒内圧縮圧力を通常時よりも低減する遅角側の所定タイミングとなる位置で前記開閉タイミング手段変更手段を固定可能な固定手段と、
前記固定手段の状態を検出する検出手段と、
所定のアイドル条件が成立し前記検出手段によって前記固定手段が前記所定タイミングとなる位置での固定を解除した状態であると検出されたときには前記所定タイミングよりも進角した位置で前記内燃機関をアイドル運転する通常時アイドル制御を実行するよう前記内燃機関と前記開閉タイミング変更手段とを制御し、所定のアイドル条件が成立し前記検出手段によって前記固定手段が前記所定タイミングとなる位置での固定を解除できない状態であると検出されたときには前記通常時アイドル制御と異なる固定時制御を実行するよう前記内燃機関を制御する制御手段と、
を備えたものである。

【０００７】
この内燃機関装置では、所定のアイドル条件が成立し固定手段が筒内圧縮圧力を通常時よりも低減する遅角側の所定タイミングとなる位置での固定を解除した状態であるときにはこの所定タイミングよりも進角した位置で内燃機関をアイドル運転する通常時アイドル制御を実行するよう内燃機関と開閉タイミング変更手段とを制御し、所定のアイドル条件が成立し固定手段が所定タイミングとなる位置での固定を解除できない状態であるときには通常時アイドル制御と異なる固定時制御を実行するよう内燃機関を制御する。ここで、開閉タイミング変更手段の固定を解除可能であるときには遅角側の所定タイミングよりも進角させてアイドル運転を実行することにより内燃機関の振動が抑えられるが、固定手段が筒内圧縮圧力を通常時よりも低減した遅角側の所定タイミングとなる位置での固定を解除できないときには、圧縮される筒内の気体量が少ないことから内燃機関からアイドル回転数を安定して維持可能な動力を出力できないことがあり、この状態で内燃機関のアイドル運転を行うと、振動が生じることがある。ここでは、固定手段が遅角側の所定タイミングとなる位置での固定を解除できない状態で通常時アイドル制御を実行してしまうのを固定時制御を行うことによって回避するのである。したがって、固定手段が遅角側の所定タイミングとなる位置での固定を解除できないときに、通常時アイドル制御を実行することにより生じる内燃機関の振動を抑制することができる。
【０００８】
本発明の内燃機関装置は、駆動軸に動力の入出力が可能な電動機と、前記内燃機関の出力軸と前記駆動軸とに接続され、電力と動力との入出力を伴って該内燃機関からの動力の少なくとも一部を前記駆動軸に出力可能な電力動力入出力手段と、前記電動機と前記電力動力入出力手段と電力のやり取りが可能な蓄電手段と、を備えたものとしてもよい。このとき、前記電力動力入出力手段は、前記駆動軸と前記内燃機関の出力軸と回転可能な回転軸の３軸に接続され該３軸のうちのいずれか２軸に入出力される動力に基づいて残余の軸に動力を入出力する３軸式動力入出力手段と、前記回転軸に動力を入出力可能な発電機と、を備える手段であるものとしてもよい。
【０００９】
本発明の内燃機関装置において、前記制御手段は、前記固定時制御を実行するに際して、前記蓄電手段に電力を蓄電させるよう前記内燃機関と前記電動機と前記電力動力入出力手段とを制御する蓄電制御を実行するものとしてもよい。こうすれば、蓄電制御を利用して電動機や電力動力入出力手段の駆動を伴って内燃機関から出力する動力を高めるため、内燃機関の振動の発生を抑制しやすい。あるいは、前記制御手段は、前記固定時制御を実行するに際して、前記蓄電手段に電力を蓄電させる蓄電制御と前記内燃機関の運転停止とを前記蓄電手段の残容量に基づいて切り替えて実行するよう前記内燃機関と前記電動機と前記電力動力入出力手段とを制御するものとしてもよい。このとき、前記制御手段は、前記蓄電制御と前記内燃機関の運転停止とを前記蓄電手段の残容量に基づいて切り替えて実行するに際して、前記蓄電手段の残容量が所定値未満であるときには前記蓄電制御を実行するよう前記内燃機関と前記電動機と前記電力動力入出力手段とを制御し、前記蓄電手段の残容量が前記所定値以上であるときには前記内燃機関の運転を停止させるものとしてもよい。こうすれば、蓄電手段の残容量が所定値より小さいときには蓄電制御を利用して電動機や電力動力入出力手段の駆動を伴って内燃機関から出力する動力を高めることが可能であり、蓄電手段の残容量が所定値以上であるときにはアイドル条件が成立していても内燃機関を停止するため、蓄電手段の保護を図ると共に内燃機関の振動の発生を抑制することができる。
【００１０】
本発明の内燃機関装置において、前記制御手段は、前記固定時制御を実行するに際して、前記内燃機関の運転を停止させるものとしてもよい。こうすれば、開閉タイミング変更手段の遅角側の所定タイミングでの固定を解除できないときにはアイドル条件が成立していても内燃機関を停止するため、内燃機関の振動の発生を防止することができる。
【００１１】

本発明の内燃機関装置において、前記制御手段は、前記固定時制御を実行するに際して、前記通常時アイドル制御よりも高い回転数で前記内燃機関をアイドル運転するよう前記内燃機関を制御するものとしてもよい。こうすれば、開閉タイミング変更手段の遅角側の所定タイミングでの固定を解除できない状態で内燃機関の通常時アイドル制御を行う場合に比べてトルク変動が小さくなるため、内燃機関の振動の発生を抑制することができる。

【００１２】

本発明の車両は、上述したいずれかに記載の内燃機関装置を搭載し、車軸が前記駆動軸に連結されてなるものである。この内燃機関装置は、上述したように吸気バルブの開閉タイミングを変更可能な内燃機関のアイドル運転において振動の発生を抑制することができるものであるから、これを備えた車両も同様の効果が得られる。

【００１３】

本発明の内燃機関装置の制御方法は、

内燃機関と、前記内燃機関の吸気バルブの開閉タイミングを変更可能な開閉タイミング変更手段と、吸気バルブの開閉タイミングが通常時よりもコンプレッション仕事を低減する遅角側の所定タイミングとなる位置で前記開閉タイミング手段変更手段を固定可能な固定手段とを備えた内燃機関装置の制御方法であって、

所定のアイドル条件が成立し前記固定手段が前記所定タイミングとなる位置での固定を解除した状態であるときには前記所定タイミングよりも進角した位置で前記内燃機関をアイドル運転する通常時アイドル制御を実行するよう前記内燃機関と前記開閉タイミング変更手段とを制御し、所定のアイドル条件が成立し前記固定手段が前記所定タイミングとなる位置での固定を解除できない状態であるときには前記通常時アイドル制御と異なる固定時制御を実行するよう前記内燃機関を制御する、

ことを含むものである。

【００１４】

この内燃機関装置の制御方法では、所定のアイドル条件が成立し固定手段が筒内圧縮圧力を通常時よりも低減する遅角側の所定タイミングとなる位置での固定を解除した状態であるときにはこの所定タイミングよりも進角した位置で内燃機関をアイドル運転する通常時アイドル制御を実行するよう内燃機関と開閉タイミング変更手段とを制御し、所定のアイドル条件が成立し固定手段が所定タイミングとなる位置での固定を解除できない状態であるときには通常時アイドル制御と異なる固定時制御を実行するよう内燃機関を制御する。ここで、開閉タイミング変更手段の固定を解除可能であるときには遅角側の所定タイミングよりも進角させてアイドル運転を実行することにより内燃機関の振動が抑えられるが、固定手段が筒内圧縮圧力を通常時よりも低減した遅角側の所定タイミングとなる位置での固定を解除できないときには、圧縮される筒内の気体量が少ないことから内燃機関からアイドル回転数を安定して維持可能な動力を出力できないことがあり、この状態で内燃機関のアイドル運転を行うと、振動が生じることがある。ここでは、固定手段が遅角側の所定タイミングとなる位置での固定を解除できない状態で通常時アイドル制御を実行してしまうのを固定時制御を行うことによって回避するのである。したがって、固定手段が遅角側の所定タイミングとなる位置での固定を解除できないときに、通常時アイドル制御を実行することにより生じる内燃機関の振動を抑制することができる。なお、この内燃機関装置の制御方法において、上述した内燃機関装置の種々の態様を採用してもよいし、また、上述した内燃機関装置の各機能を実現するようなステップを追加してもよい。

【発明を実施するための最良の形態】

【００１５】

次に、本発明を実施するための最良の形態を実施例を用いて説明する。

【実施例】

【００１６】

図１は、本発明の一実施例である動力出力装置を搭載したハイブリッド自動車２０の構成の概略を示す構成図である。実施例のハイブリッド自動車２０は、図示するように、エンジン２２と、エンジン２２の出力軸としてのクランクシャフト２６にダンパ２８を介して接続された３軸式の動力分配統合機構３０と、動力分配統合機構３０に接続された発電可能なモータＭＧ１と、動力分配統合機構３０に接続された駆動軸としてのリングギヤ軸３２ａに取り付けられた減速ギヤ３５と、この減速ギヤ３５に接続されたモータＭＧ２と、動力出力装置全体をコントロールするハイブリッド用電子制御ユニット７０とを備える。

【００１７】

エンジン２２は、例えばガソリンまたは軽油などの炭化水素系の燃料により動力を出力可能な内燃機関として構成されており、図２に示すように、エアクリーナ１２２により清浄された空気をスロットルバルブ１２４を介して吸入する共に燃料噴射弁１２６からガソリンを噴射して吸入された空気とガソリンとを混合し、この混合気を吸気バルブ１２８を介して燃料室に吸入し、点火プラグ１３０による電気火花によって爆発燃焼させて、そのエネルギにより押し下げられるピストン１３２の往復運動をクランクシャフト２３の回転運動に変換する。エンジン２２からの排気は、一酸化炭素（ＣＯ）や炭化水素（ＨＣ），窒素酸化物（ＮＯｘ）の有害成分を浄化する浄化装置（三元触媒）１３４を介して外気へ排出される。

【００１８】

また、エンジン２２は、吸気バルブ１２８の開閉タイミングを連続的に変更可能な可変バルブタイミング機構１５０を備える。図３および図４に、可変バルブタイミング機構１５０の構成の概略を示す構成図を示す。可変バルブタイミング機構１５０は、図示するように、クランクシャフト２６にタイミングチェーン１６２を介して接続されたタイミングギヤ１６４に固定されたハウジング部１５２ａと吸気バルブ１２８を開閉するインテークカムシャフト１２９に固定されたベーン部１５２ｂとからなるベーン式のＶＶＴコントローラ１５２と、ベーン部１５２ｂの位置を検出するベーンポジションセンサ１５３と、ＶＶＴコントローラ１５２の進角室および遅角室に油圧を作用させるオイルコントロールバルブ１５６とを備え、オイルコントロールバルブ１５６を介してＶＶＴコントローラ１５２の進角室および遅角室に作用させる油圧を調節することによりハウジング部１５２ａに対してベーン部１５２ｂを相対的に回転させて吸気バルブ１２８の開閉タイミングにおけるインテークカムシャフト１２９の角度を連続的に変更する。インテークカムシャフト１２９の角度を進角させたときの吸気バルブ１２８の開閉タイミングおよびインテークカムシャフト１２９の角度を遅角させたときの吸気バルブ１２８の開閉タイミングの一例を図５に示す。実施例では、エンジン２２から効率よく動力が出力される吸気バルブ１２８の開閉タイミングにおけるインテークカムシャフト１２９の角度を基準角とし、インテークカムシャフト１２９の角度をその基準角よりも進角させることによりエンジン２２から高トルクが出力可能な運転状態とすることができ、インテークカムシャフト１２９の角度を最遅角させることによりエンジン２２の気筒内の圧力変動を小さくしてエンジン２２の運転の停止や始動に適した運転状態とすることができるよう構成されている。

【００１９】

また、ＶＶＴコントローラ１５２のベーン部１５２ｂには、ハウジング部１５２ａとベーン部１５２ｂとの相対回転を固定するロックピン１５４が取り付けられている。図６にロックピン１５４の構成の概略を示す構成図を示す。ロックピン１５４は、図示するようにロックピン本体１５４ａと、ロックピン本体１５４ａがハウジング部１５２ａの方向に付勢されるよう取り付けられたスプリング１５４ｂとを備え、インテークカムシャフト１２９の角度が最遅角に位置されたときにスプリング１５４ｂのスプリング力によりハウジング部１５２ａに形成された溝１５８に嵌合しベーン部１５２ｂをハウジング部１５２ａに固定する。また、ロックピン１５４は、油路１５９を介してスプリング１５４ｂのスプリング力に打ち勝つ油圧を作用させることにより溝１５８に嵌合されたロックピン本体１５４ａを引き抜くことができるよう図示しない油圧式のアクチュエータが設けられている。

【００２０】

エンジン２２は、エンジン用電子制御ユニット（以下、エンジンＥＣＵという）２４に

より制御されている。エンジンＥＣＵ２４は、ＣＰＵ２４ａを中心とするマイクロプロセッサとして構成されており、ＣＰＵ２４ａの他に処理プログラムを記憶するＲＯＭ２４ｂと、データを一時的に記憶するＲＡＭ２４ｃと、図示しない入出力ポートおよび通信ポートとを備える。エンジンＥＣＵ２４には、エンジン２２の状態を検出する種々のセンサからの信号、クランクシャフト２３の回転位置を検出するクランクポジションセンサ１４０からのクランクポジションやエンジン２２の冷却水の温度を検出する水温センサ１４２からの冷却水温，燃焼室内に取り付けられた圧力センサ１４３からの筒内圧力Ｐｉｎ，燃焼室へ吸排気を行なう吸気バルブ１２８や排気バルブを開閉するカムシャフトの回転位置を検出するカムポジションセンサ１４４からのカムポジション，スロットルバルブ１２４のポジションを検出するスロットルバルブポジションセンサ１４６からのスロットルポジション，吸気管に取り付けられたエアフローメータ１４８からのエアフローメータ信号ＡＦ，同じく吸気管に取り付けられた温度センサ１４９からの吸気温，空燃比センサ１３５ａからの空燃比ＡＦ，酸素センサ１３５ｂからの酸素信号，ベーンポジションセンサ１５３からのベーンポジションなどが入力ポートを介して入力されている。また、エンジンＥＣＵ２４からは、エンジン２２を駆動するための種々の制御信号、例えば、燃料噴射弁１２６への駆動信号や、スロットルバルブ１２４のポジションを調節するスロットルモータ１３６への駆動信号、イグナイタと一体化されたイグニッションコイル１３８への制御信号、吸気バルブ１２８の開閉タイミングの変更可能な可変バルブタイミング機構１５０への制御信号などが出力ポートを介して出力されている。なお、エンジンＥＣＵ２４は、ハイブリッド用電子制御ユニット７０と通信しており、ハイブリッド用電子制御ユニット７０からの制御信号によりエンジン２２を運転制御すると共に必要に応じてエンジン２２の運転状態に関するデータを出力する。

【００２１】

動力分配統合機構３０は、外歯歯車のサンギヤ３１と、このサンギヤ３１と同心円上に配置された内歯歯車のリングギヤ３２と、サンギヤ３１に噛合すると共にリングギヤ３２に噛合する複数のピニオンギヤ３３と、複数のピニオンギヤ３３を自転かつ公転自在に保持するキャリア３４とを備え、サンギヤ３１とリングギヤ３２とキャリア３４とを回転要素として差動作用を行なう遊星歯車機構として構成されている。動力分配統合機構３０は、キャリア３４にはエンジン２２のクランクシャフト２６が、サンギヤ３１にはモータＭＧ１が、リングギヤ３２にはリングギヤ軸３２ａを介して減速ギヤ３５がそれぞれ連結されており、モータＭＧ１が発電機として機能するときにはキャリア３４から入力されるエンジン２２からの動力をサンギヤ３１側とリングギヤ３２側にそのギヤ比に応じて分配し、モータＭＧ１が電動機として機能するときにはキャリア３４から入力されるエンジン２２からの動力とサンギヤ３１から入力されるモータＭＧ１からの動力を統合してリングギヤ３２側に出力する。リングギヤ３２に出力された動力は、リングギヤ軸３２ａからギヤ機構６０およびデファレンシャルギヤ６２を介して、最終的には車両の駆動輪６３ａ，６３ｂに出力される。

【００２２】

モータＭＧ１およびモータＭＧ２は、いずれも発電機として駆動することができると共に電動機として駆動できる周知の同期発電電動機として構成されており、インバータ４１，４２を介してバッテリ５０と電力のやりとりを行なう。インバータ４１，４２とバッテリ５０とを接続する電力ライン５４は、各インバータ４１，４２が共用する正極母線および負極母線として構成されており、モータＭＧ１，ＭＧ２のいずれかで発電される電力を他のモータで消費することができるようになっている。したがって、バッテリ５０は、モータＭＧ１，ＭＧ２のいずれかから生じた電力や不足する電力により充放電されることになる。なお、モータＭＧ１，ＭＧ２により電力収支のバランスをとるものとすれば、バッテリ５０は充放電されない。モータＭＧ１，ＭＧ２は、いずれもモータ用電子制御ユニット（以下、モータＥＣＵという）４０により駆動制御されている。モータＥＣＵ４０には、モータＭＧ１，ＭＧ２を駆動制御するために必要な信号、例えばモータＭＧ１，ＭＧ２の回転子の回転位置を検出する回転位置検出センサ４３，４４からの信号や図示しない電

流センサにより検出されるモータＭＧ１，ＭＧ２に印加される相電流などが入力されており、モータＥＣＵ４０からは、インバータ４１，４２へのスイッチング制御信号が出力されている。モータＥＣＵ４０は、ハイブリッド用電子制御ユニット７０と通信しており、ハイブリッド用電子制御ユニット７０からの制御信号によってモータＭＧ１，ＭＧ２を駆動制御すると共に必要に応じてモータＭＧ１，ＭＧ２の運転状態に関するデータをハイブリッド用電子制御ユニット７０に出力する。

【００２３】

バッテリ５０は、バッテリ用電子制御ユニット（以下、バッテリＥＣＵという）５２によって管理されている。バッテリＥＣＵ５２には、バッテリ５０を管理するのに必要な信号、例えば、バッテリ５０の端子間に設置された図示しない電圧センサからの端子間電圧，バッテリ５０の出力端子に接続された電力ライン５４に取り付けられた図示しない電流センサからの充放電電流，バッテリ５０に取り付けられた温度センサ５１からの電池温度Ｔｂなどが入力されており、必要に応じてバッテリ５０の状態に関するデータを通信によりハイブリッド用電子制御ユニット７０に出力する。なお、バッテリＥＣＵ５２では、バッテリ５０を管理するために電流センサにより検出された充放電電流の積算値に基づいて残容量（ＳＯＣ）も演算している。

【００２４】

ハイブリッド用電子制御ユニット７０は、ＣＰＵ７２を中心とするマイクロプロセッサとして構成されており、ＣＰＵ７２の他に処理プログラムを記憶するＲＯＭ７４と、データを一時的に記憶するＲＡＭ７６と、図示しない入出力ポートおよび通信ポートとを備える。ハイブリッド用電子制御ユニット７０には、イグニッションスイッチ８０からのイグニッション信号，シフトレバー８１の操作位置を検出するシフトポジションセンサ８２からのシフトポジションＳＰ，アクセルペダル８３の踏み込み量を検出するアクセルペダルポジションセンサ８４からのアクセル開度Ａｃｃ，ブレーキペダル８５の踏み込み量を検出するブレーキペダルポジションセンサ８６からのブレーキペダルポジションＢＰ，車速センサ８８からの車速Ｖなどが入力ポートを介して入力されている。ハイブリッド用電子制御ユニット７０は、前述したように、エンジンＥＣＵ２４やモータＥＣＵ４０，バッテリＥＣＵ５２と通信ポートを介して接続されており、エンジンＥＣＵ２４やモータＥＣＵ４０，バッテリＥＣＵ５２と各種制御信号やデータのやりとりを行なっている。

【００２５】

こうして構成された実施例のハイブリッド自動車２０は、運転者によるアクセルペダル８３の踏み込み量に対応するアクセル開度Ａｃｃと車速Ｖとに基づいて駆動軸としてのリングギヤ軸３２ａに出力すべき要求トルクを計算し、この要求トルクに対応する要求動力がリングギヤ軸３２ａに出力されるように、エンジン２２とモータＭＧ１とモータＭＧ２とが運転制御される。エンジン２２とモータＭＧ１とモータＭＧ２の運転制御としては、要求動力に見合う動力がエンジン２２から出力されるようにエンジン２２を運転制御すると共にエンジン２２から出力される動力のすべてが動力分配統合機構３０とモータＭＧ１とモータＭＧ２とによってトルク変換されてリングギヤ軸３２ａに出力されるようモータＭＧ１およびモータＭＧ２を駆動制御するトルク変換運転モードや要求動力とバッテリ５０の充放電に必要な電力との和に見合う動力がエンジン２２から出力されるようにエンジン２２を運転制御すると共にバッテリ５０の充放電を伴ってエンジン２２から出力される動力の全部またはその一部が動力分配統合機構３０とモータＭＧ１とモータＭＧ２とによるトルク変換を伴って要求動力がリングギヤ軸３２ａに出力されるようモータＭＧ１およびモータＭＧ２を駆動制御する充放電運転モード、エンジン２２の運転を停止してモータＭＧ２からの要求動力に見合う動力をリングギヤ軸３２ａに出力するよう運転制御するモータ運転モードなどがある。

【００２６】

ハイブリッド自動車２０は、車両に要求される要求パワーなどに基づいてエンジン２２の運転・停止を繰り返す間欠運転を行う。このハイブリッド自動車２０は、エンジン２２を停止する際には、可変バルブタイミング機構１５０を駆動してロックピン１５４を溝１

５８に嵌合させることによりベーン部１５２ｂを最遅角位置で固定させるよう設定されている。また、ハイブリッド自動車２０は、エンジン２２を始動する際には、ロックピン１５４によりベーン部１５２ｂを最遅角位置で固定し圧縮行程の筒内圧縮圧力を通常時よりも低減した状態でモータＭＧ１によりクランキングを行い、エンジン２２が完爆したあとに油圧をロックピン本体１５４ａに作用させ溝１５８への嵌合を解除し、最遅角位置よりも進角側の吸気バルブ１２８の開閉タイミングでエンジン２２の運転を行うよう設定されている。このように、エンジン２２の始動時には、吸気バルブ１２８の開閉タイミングを最遅角位置とし筒内圧縮圧力を低減するからエンジン２２を始動しやすいし、ロックピン１５４によりハウジング部１５２ａとベーン部１５２ｂとを固定するからこれらの間のがたつきを防止することができる。

【００２７】

次に、こうして構成された実施例のハイブリッド自動車２０の動作、特にエンジン２２のアイドル運転を実行する際の動作について説明する。図７は、エンジンＥＣＵ２４により実行されるアイドル要求時制御ルーチンの一例を示すフローチャートである。このルーチンは、エンジンＥＣＵ２４がハイブリッド用電子制御ユニット７０からアイドル指令を受信している間に所定時間毎（例えば数ｍｓｅｃ毎）に繰り返し実行される。このアイドル指令は、車両に要求される要求パワーＰ＊が所定の閾値Ｐｒｅｆよりも小さい場合などエンジンからのパワーの出力が要求されていないときや、エンジン２２の暖機時などのアイドル条件が成立したときなどにハイブリッド用電子制御ユニット７０から送信される。以下、説明の便宜のため、シフトポジションＳＰがパーキングポジションにありエンジン２２の暖機時について説明する。

【００２８】

アイドル要求時制御ルーチンが実行されると、エンジンＥＣＵ２４のＣＰＵ２４ａは、まず、バッテリ５０の残容量ＳＯＣをバッテリＥＣＵ５２から入力する（ステップＳ１００）。次に、油圧を用いてロックピン１５４の嵌合を解除すると共に予め定められたアイドル運転用の開閉タイミングとなるよう可変バルブタイミング機構１５０を駆動し（ステップＳ１１０）、ロックピン１５４によるハウジング部１５２ａとベーン部１５２ｂとの間の固定が解除されているか否かを判定する（ステップＳ１２０）。ここで、アイドル運転用の開閉タイミングとなるベーン部１５２ｂの位置は、本実施例では、基準角となる位置（図５参照）の数度遅角側の位置に設定されている。また、ロックピン１５４が解除されているか否かの判定は、可変バルブタイミング機構１５０を駆動したときにベーン部１５２ｂの位置が最遅角位置から変化したか否かをベーンポジションセンサ１５３から入力したベーンポジションに基づいて判定することにより行う。このロックピン１５４は、例えばロックピン１５４を作動するアクチュエータの油温が高くなることにより作動油の粘性が低くなると油圧がロックピン本体１５４ａに作用しにくくなり溝１５８から抜けにくくなることがある。ロックピン１５４によるベーン部１５２ｂの固定が解除できないときは、最遅角の開閉タイミングで吸気バルブ１２８が開閉することになる。なお、可変バルブタイミング機構１５０が既にアイドル運転用の開閉タイミングとなっているときには、ステップＳ１１０の処理は省略される。

【００２９】

ステップＳ１２０でロックピン１５４による固定が解除されていると判定されたときには、可変バルブタイミング機構１５０がアイドル運転用の開閉タイミングとなるのを待って、アイドル制御を実行し（ステップＳ１３０）、このルーチンを終了する。ここで、アイドル制御としては、エンジン２２の回転数Ｎｅが所定のアイドル回転数Ｎｅｉｄｌ（例えば６００ｒｐｍや１０００ｒｐｍなど）となるようにスロットルバルブ１２４のポジションを調節する処理などを行う。

【００３０】

一方、ステップＳ１２０でロックピン１５４による固定が解除されていないと判定されたときには、通常のアイドル制御とは異なるロックピン固定時制御を実行する（ステップＳ１４０～Ｓ１６０）。具体的には、まず、ステップＳ１００で入力したバッテリ５０の

残容量ＳＯＣが閾値Ｓ１未満であるか否かを判定する（ステップＳ１４０）。この閾値Ｓ１は、バッテリ５０に電力を充電可能か否かを判定する値であり、残容量ＳＯＣの最大値の７０％や８０％などに定めることができる。バッテリ５０の残容量ＳＯＣが閾値Ｓ１未満であると判定されたときには、バッテリ５０へ電力を充電可能であるものとみなし、バッテリ５０の充電制御を実行し（ステップＳ１５０）、このルーチンを終了する。

【００３１】

ここでバッテリ充電制御について説明する。このバッテリ充電制御は、エンジン目標回転数Ｎｅ＊を所定の充電用回転数Ｎｅｃｈに設定すると共にエンジン目標トルクＴｅ＊を所定の充電用トルクＴｅｃｈに設定し、この目標回転数Ｎｅ＊，目標トルクＴｅ＊がエンジン２２から出力されるようモータＭＧ１の駆動制御を行うのである。ここでは、エンジン２２の充電用回転数Ｎｅｃｈは、アイドル回転数Ｎｅｉｄｌよりも高い回転数（例えば１５００ｒｐｍや２０００ｒｐｍなど）に設定されている。また、モータＭＧ１の駆動制御は、エンジンＥＣＵ２４がハイブリッド用電子制御ユニット７０に充電指令を送信し、この充電指令を受信したハイブリッド用電子制御ユニット７０がモータＭＧ１の目標回転数Ｎｍ１＊とトルク指令Ｔｍ１とを設定し、この設定された目標回転数Ｎｍ１＊とトルク指令Ｔｍ１とになるようにインバータ４１を制御することにより行われる。ここで、動力分配統合機構３０の回転要素における回転数とトルクとの力学的な関係を示す共線図を図８に示す。図中、左のＳ軸はモータＭＧ１の回転数Ｎｍ１であるサンギヤ３１の回転数を示し、Ｃ軸はエンジン２２の回転数Ｎｅであるキャリア３４の回転数を示し、Ｒ軸はモータＭＧ２の回転数Ｎｍ２を減速ギヤ３５のギヤ比Ｇｒで除したリングギヤ３２の回転数Ｎｒを示し、「ρ」は、動力分配統合機構３０のギヤ比を示す。図８に示すように、モータＭＧ１を回転数Ｎｍ１，トルクＴｍ１で駆動すると、それに応じた電力がバッテリ５０に充電される。

【００３２】

ここで、アイドル運転ではエンジン２２の回転数Ｎｅが比較的小さいことや、筒内圧縮圧力を低減する最遅角位置でベーン部１５２ｂが固定された状態では圧縮行程で圧縮する混合気量が通常時のアイドリング制御に比べて少なくエンジン２２からトルクを出力しにくくエンジン２２の回転数Ｎｅが不安定になることなどがあるため、このような状態でアイドル運転を行うと振動や騒音が発生してしまう。ここでは、アイドル回転数Ｎｅｉｄｌよりも高い回転数Ｎｅｃｈで、且つモータＭＧ１によってエンジン２２の回転を押さえ込む方向にトルクをかけてエンジン２２からトルクをより出力する状態（発電状態）とするため、振動や騒音を低減することができる。このように、ロックピン１５４が筒内圧縮圧力を低減する最遅角位置でのベーン部１５２ｂの固定を解除できずバッテリ５０が充電可能であるときには、エンジン２２のアイドル条件が成立していてもバッテリ５０への充電制御を実行するのである。

【００３３】

一方、ステップＳ１４０でバッテリ５０の残容量ＳＯＣが閾値Ｓ１以上であると判定されたときには、バッテリ５０へ電力を充電できないものとみなし、エンジン２２を停止させ（ステップＳ１６０）、このルーチンを終了する。ここで、エンジン２２の停止は、燃料噴射弁１２６からの燃料噴射を停止することによりフリクションを利用してエンジン２２の回転数Ｎｅを低下させる。このように、ロックピン１５４が筒内圧縮圧力を低減する最遅角位置でのベーン部１５２ｂの固定を解除できずバッテリ５０が充電できないときには、エンジン２２のアイドル条件が成立していてもエンジン２２を停止するのである。

【００３４】

その後、ロックピン１５４を作動させるアクチュエータの作動油の温度が低下するなどしてロックピン本体１５４ａに油圧がかかるようになると、ロックピン１５４によるハウジング部１５２ａとベーン部１５２ｂとの固定が解除されて可変バルブタイミング機構１５０が自由に駆動可能となる。そして、アイドル条件が成立したときには、上述したアイドル要求時制御ルーチンのアイドル制御を実行する（ステップＳ１００～Ｓ１３０）。

【００３５】

以上詳述した本実施例のハイブリッド自動車２０によれば、所定のアイドル条件が成立しロックピン１５４が筒内圧縮圧力の低い最遅角位置での固定を解除した状態であるときにはこの最遅角位置よりも進角した位置でエンジン２２をアイドル運転し、所定のアイドル条件が成立しロックピン１５４が最遅角位置での固定を解除できない状態であり、バッテリ５０の残容量ＳＯＣが閾値Ｓ１未満であるときにはバッテリ５０の充電制御を実行するようエンジン２２とモータＭＧ１，ＭＧ２とを制御し、バッテリ５０の残容量ＳＯＣが閾値Ｓ１以上であるときにはエンジン２２を停止させる。ここで、可変バルブタイミング機構１５０の固定を解除可能であるときには最遅角位置よりも進角させてアイドル制御を実行することによりエンジン２２の振動が抑えられるが、ロックピン１５４が筒内圧縮圧力を通常時よりも低減した最遅角位置での固定を解除できないときには、圧縮される筒内の空気量が少ないことからエンジン２２からアイドル回転数Ｎｅｉｄｌを安定して維持可能な動力を出力できないことがあり、この状態でエンジン２２のアイドル運転を行うと振動が生じてしまう。ここでは、ロックピン１５４が最遅角位置での固定を解除できない状態で通常時アイドル制御を実行してしまうのをロックピン固定時制御を行うことによって回避するのである。このように、可変バルブタイミング機構１５０の最遅角位置での固定を解除できないときには、モータＭＧ１，ＭＧ２の駆動を伴ってバッテリ５０を充電することによりエンジン２２から出力する動力を高めるため、エンジン２２の振動の発生を抑制しやすいし、アイドル条件が成立していてもエンジン２２を停止するため、エンジン２２の振動の発生を防止することができる。また、バッテリ５０の残容量ＳＯＣに基づいてバッテリ５０の充電制御とエンジン２２の停止とを切り替えるため、バッテリ５０を十分に保護することができる。また、バッテリ５０の充電制御において通常のアイドル回転数Ｎｅｉｄｌよりも高い充電用回転数Ｎｅｃｈでエンジン２２を運転するため、筒内圧縮圧力が小さい状態で通常時のエンジン２２の回転数Ｎｅｉｄｌでアイドル制御する場合に比べてトルク変動が小さくなり、エンジン２２の振動の発生を抑制しやすい。

【００３６】

なお、本発明は上述した実施例に何ら限定されることはなく、本発明の技術的範囲に属する限り種々の態様で実施し得ることはいうまでもない。

【００３７】

例えば、上述した実施例では、バッテリ５０の残容量ＳＯＣに基づいてバッテリ５０の充電制御の実行とエンジン２２の停止とを切り替えるものとしたが、バッテリ５０の充電制御とエンジン２２の停止との少なくとも一方を実行するものとしてもよい。こうしても、エンジン２２のアイドル運転において振動の発生を抑制することはできる。

【００３８】

上述した実施例では、バッテリ５０の残容量ＳＯＣに基づいてバッテリ５０の充電制御とエンジン２２の停止とを切り替えるものとしたが、このバッテリ５０の残容量ＳＯＣを温度センサ５１による電池温度Ｔｂを加味したものに基づいてバッテリ５０の充電制御とエンジン２２の停止とを切り替えるものとしてもよい。こうすれば、バッテリ５０の温度状態を用いてバッテリ５０の保護を一層図ることができる。

【００３９】

上述した実施例では、エンジン２２の目標回転数Ｎｅ＊をエンジン２２のアイドル回転数Ｎｅｉｄｌよりも高い回転数Ｎｅｃｈとしてバッテリ５０の充電制御を実行するものとしたが、エンジン２２の目標回転数Ｎｅ＊をエンジン２２のアイドル回転数Ｎｅｉｄｌとして充電制御を実行するものとしてもよい。こうしても、エンジン２２から動力が出力されることによって、エンジン２２の振動を低減することができる。

【００４０】

上述した実施例では、アイドル条件が成立しロックピン１５４が最遅角位置での固定を解除できない状態である場合には、バッテリ５０の充電制御とエンジン２２の停止とを実行するものとしたが、これに代えて、通常時のアイドル制御のエンジン２２の回転数Ｎｅｉｄｌよりも高い回転数でエンジン２２をアイドル制御するものとしてもよい。こうすれば、筒内圧縮圧力が小さい状態で通常時のエンジン２２の回転数Ｎｅｉｄｌでアイドル制御する場合に比べてトルク変動が小さくなるため、エンジン２２の振動の発生を抑制することができる。このとき、エンジン２２の回転数Ｎｅをアイドル回転数Ｎｅｉｄｌとするのに必要なアイドル制御量（スロットルバルブ１２４の開度など）を学習するアイドル学習を行わないものとしてもよい。こうすれば、アイドル制御値が通常と異なる状態で運転されている状態のものになってしまうのを防止することができる。

【００４１】

上述した実施例では、最遅角位置で可変バルブタイミング機構１５０をロックピン１５４により固定するものとしたが、最遅角位置から数度進角した位置などで可変バルブタイミング機構１５０をロックピン１５４により固定するものとしてもよい。

【００４２】

上述した実施例では、ベーン部１５２ｂの位置を検出するベーンポジションセンサ１５３によりロックピン１５４による可変バルブタイミング機構１５０の固定が解除されたか否かを検出するものとしたが、ロックピン本体１５４ａの位置を検出するロックピンポジションセンサを可変バルブタイミング機構１５０に設け、このセンサの出力値を用いてロックピン１５４による可変バルブタイミング機構１５０の固定が解除されたか否かを検出するものとしてもよい。このとき、上述のアイドル要求時制御ルーチンにおいて、ロックピン１５４の固定が解除されたか否かを判定したのちに可変バルブタイミング機構１５０の駆動を行うようにしてもよい。

【００４３】

上述した実施例では、ハイブリッド自動車２０が停車状態でありエンジン２２の暖気時にアイドル条件が成立した場合について説明したが、ハイブリッド自動車２０が走行中のときにアイドル条件が成立してもよい。このとき、ハイブリッド用電子制御ユニット７０は、駆動軸としてのリングギヤ軸３２ａに要求される車両の走行用の要求トルクＴｒ＊に、モータＭＧ１から出力された反力トルクである（－１／ρ・Ｔｍ１）のトルクを加味したトルクがモータＭＧ２から出力されるようインバータ４２などを制御すればよい。

【００４４】

上述した実施例では、モータＭＧ１，ＭＧ２を備えたハイブリッド自動車２０としたが、吸気バルブの開閉タイミングが筒内圧縮圧力を通常時よりも低減する遅角側のタイミングとなる位置で固定するロックピン１５４を有する可変バルブタイミング機構１５０を備えたエンジン２２を搭載した車両であれば特にこれに限定されず、例えば、エンジンを始動停止するアイドルストップ自動車に適用するものとしてもよい。この場合においても、上述した実施例と同様に、アイドル条件が成立しロックピン１５４が最遅角位置での固定を解除できない状態である場合には、エンジン２２を停止するものとしてもよい。こうすれば、アイドル条件が成立していてもエンジン２２を停止するため、エンジン２２の振動の発生を防止することができる。

【００４５】

実施例のハイブリッド自動車２０では、モータＭＧ２の動力を減速ギヤ３５により変速してリングギヤ軸３２ａに出力するものとしたが、図９の変形例のハイブリッド自動車１２０に例示するように、モータＭＧ２の動力をリングギヤ軸３２ａが接続された車軸（駆動輪６３ａ，６３ｂが接続された車軸）とは異なる車軸（図９における車輪６４ａ，６４ｂに接続された車軸）に接続するものとしてもよい。

【００４６】

実施例のハイブリッド自動車２０では、エンジン２２の動力を動力分配統合機構３０を介して駆動輪６３ａ，６３ｂに接続された駆動軸としてのリングギヤ軸３２ａに出力するものとしたが、図１０の変形例のハイブリッド自動車２２０に例示するように、エンジン２２のクランクシャフト２６に接続されたインナーロータ２３２と駆動輪６３ａ，６３ｂに動力を出力する駆動軸に接続されたアウターロータ２３４とを有し、エンジン２２の動力の一部を駆動軸に伝達すると共に残余の動力を電力に変換する対ロータ電動機２３０を備えるものとしてもよい。

【００４７】

上述した実施例では、エンジン２２とモータＭＧ１，ＭＧ２とを備えたシリーズ－パラレルハイブリッド自動車としたが、シリーズハイブリッド自動車としてもよいし、パラレルハイブリッド自動車としてもよい。　また、こうしたハイブリッド自動車に適用するものに限定されるものではなく、自動車以外の車両や船舶，航空機などの移動体に搭載される内燃機関装置の形態としても構わない。さらに、こうした内燃機関装置の制御方法の形態としてもよい。

【図面の簡単な説明】

【００４８】

【図１】本発明の一実施例であるハイブリッド自動車２０の構成の概略を示す構成図である。

【図２】エンジン２２の構成の概略を示す構成図である。

【図３】可変バルブタイミング機構１５０の外観構成を示す外観構成図である。

【図４】可変バルブタイミング機構１５０の構成の概略を示す構成図である。

【図５】インテークカムシャフト１２９の角度を進角させたときの吸気バルブ１２８の開閉タイミングおよびインテークカムシャフト１２９の角度を遅角させたときの吸気バルブ１２８の開閉タイミングの一例を示す説明図である。

【図６】ロックピン１５４の構成の概略を示す構成図である。

【図７】実施例のエンジンＥＣＵ２４により実行されるアイドル要求時制御ルーチンの一例を示すフローチャートである。

【図８】動力分配統合機構３０の回転要素を力学的に説明するための共線図の一例を示す説明図である。

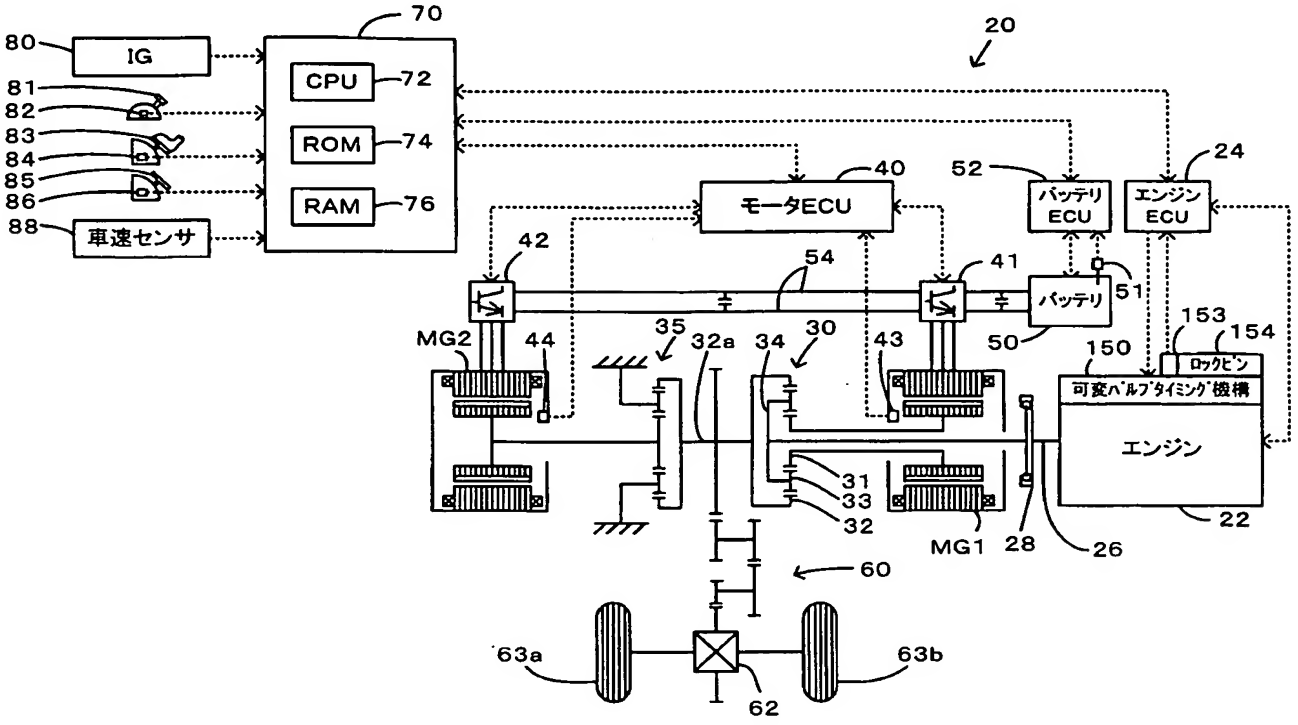
【図９】変形例のハイブリッド自動車１２０の構成の概略を示す構成図である。

【図１０】変形例のハイブリッド自動車２２０の構成の概略を示す構成図である。

【符号の説明】

【００４９】

２０，１２０，２２０　ハイブリッド自動車、２２　エンジン、２４　エンジン用電子制御ユニット（エンジンＥＣＵ）、２４ａ　ＣＰＵ、２４ｂ　ＲＯＭ、２４ｃ　ＲＡＭ、２６　クランクシャフト、２８　ダンパ、３０　動力分配統合機構、３１　サンギヤ、３２　リングギヤ、３２ａ　リングギヤ軸、３３　ピニオンギヤ、３４　キャリア、３５　減速ギヤ、４０　モータ用電子制御ユニット（モータＥＣＵ）、４１，４２　インバータ、４３，４４　回転位置検出センサ、５０　バッテリ、５１　温度センサ、５２　バッテリ用電子制御ユニット（バッテリＥＣＵ）、５４　電力ライン、６０　ギヤ機構、６２　デファレンシャルギヤ、６３ａ，６３ｂ　駆動輪、６４ａ，６４ｂ　車輪、７０　ハイブリッド用電子制御ユニット、７２　ＣＰＵ、７４　ＲＯＭ、７６　ＲＡＭ、８０　イグニッションスイッチ、８１　シフトレバー、８２　シフトポジションセンサ、８３　アクセルペダル、８４　アクセルペダルポジションセンサ、８５　ブレーキペダル、８６　ブレーキペダルポジションセンサ、８８　車速センサ、１２２　エアクリーナ、１２４　スロットルバルブ、１２６　燃料噴射弁、１２８　吸気バルブ、１２９　インテークカムシャフト、１３０　点火プラグ、１３２　ピストン、１３４　浄化装置、１３５ａ　空燃比センサ、１３６　スロットルモータ、１３８　イグニッションコイル、１４０　クランクポジションセンサ、１４２　水温センサ、１４３　圧力センサ、１４４　カムポジションセンサ、１４６　スロットルバルブポジションセンサ、１４８　エアフローメータ、１４９　温度センサ、１５０　可変バルブタイミング機構、１５２　ＶＶＴコントローラ、１５２ａ　ハウジング部、１５２ｂ　ベーン部、１５３　ベーンポジションセンサ、１５４　ロックピン、１５４ａ　ロックピン本体、１５４ｂ　スプリング、１５６　オイルコントロールバルブ、１５８　溝、１５９　油路、１６２　タイミングチェーン、１６４　タイミングギヤ、２３０　対ロータ電動機、２３２　インナーロータ　２３４　アウターロータ、ＭＧ１，ＭＧ２　モータ。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モータECU
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バッテリECU
24
エンジンECU
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54
41
バッテリ
51
153
154
ロックピン
150
可変バルブタイミング機構
エンジン
MG2
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32
MG1
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63a
63b
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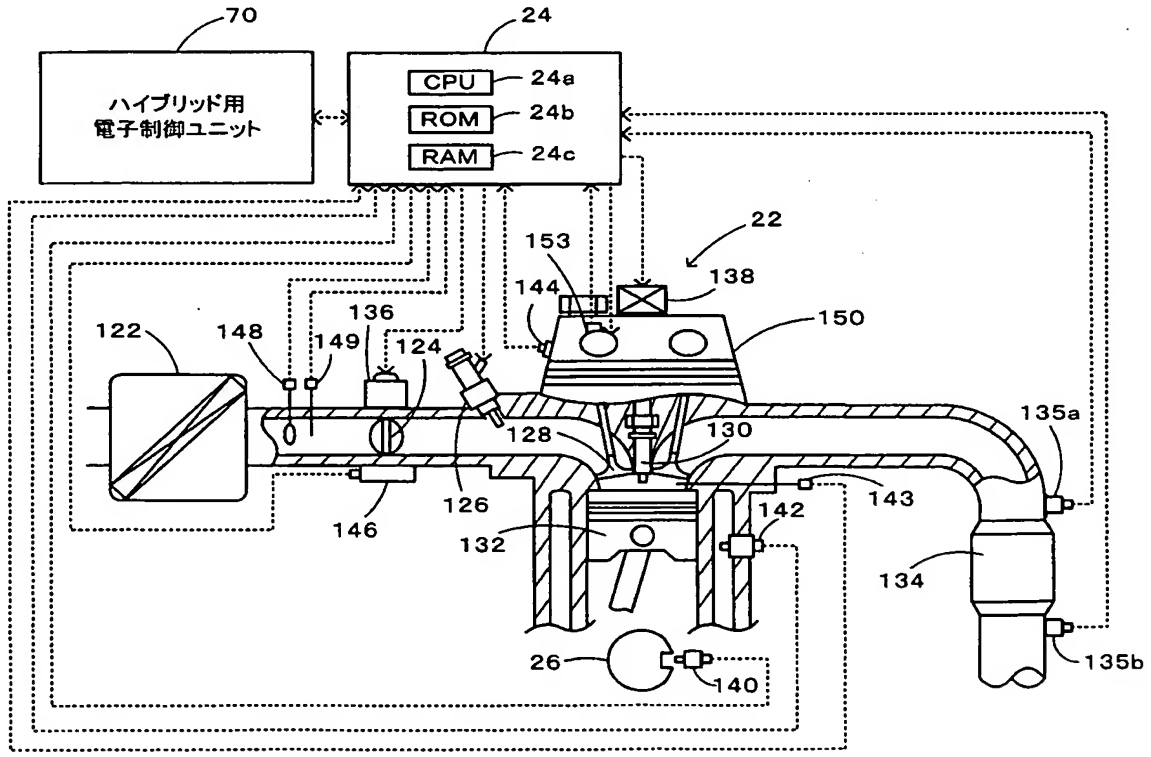
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ハイブリッド用
電子制御ユニット
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CPU
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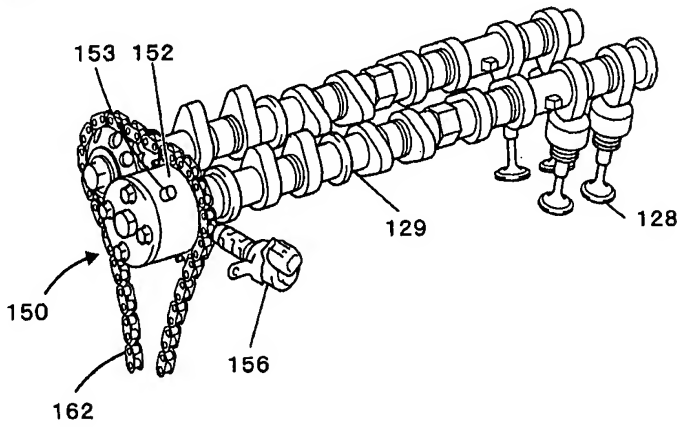
【図３】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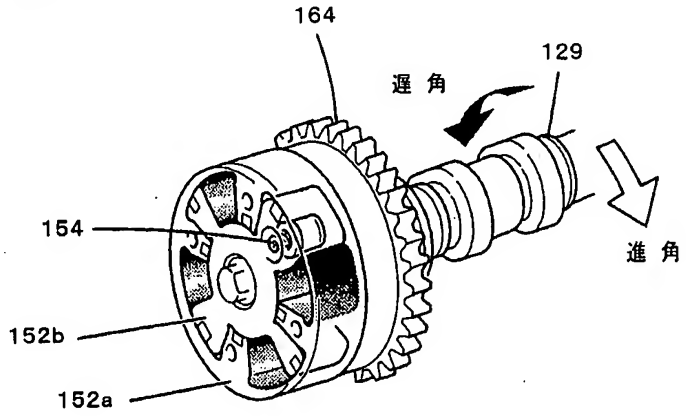
【図４】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遅 角
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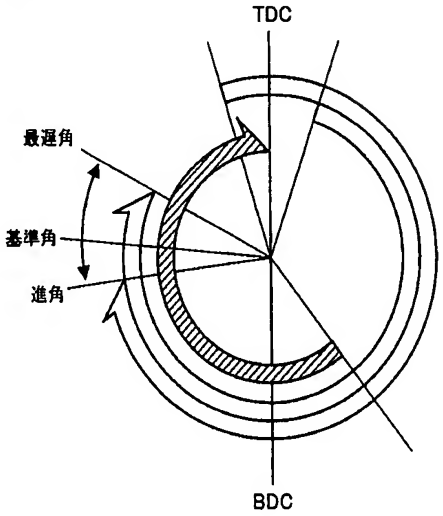
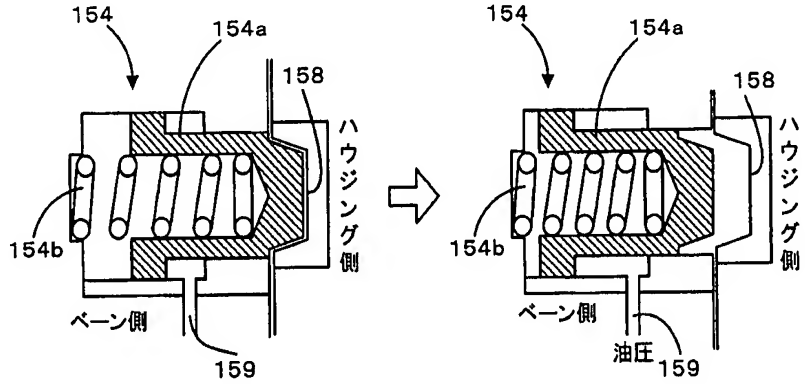
152a

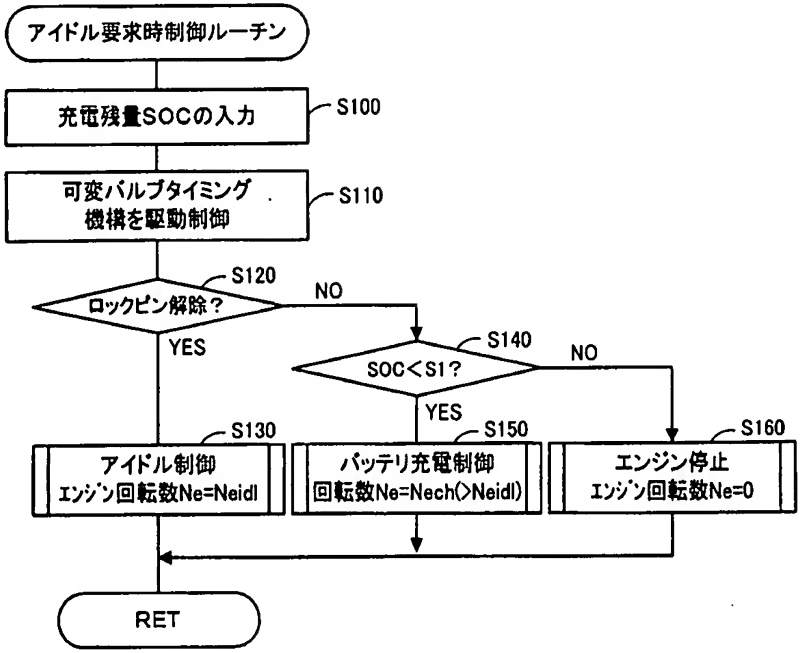
【図５】


TDC
最遅角
基準角
進角
BDC

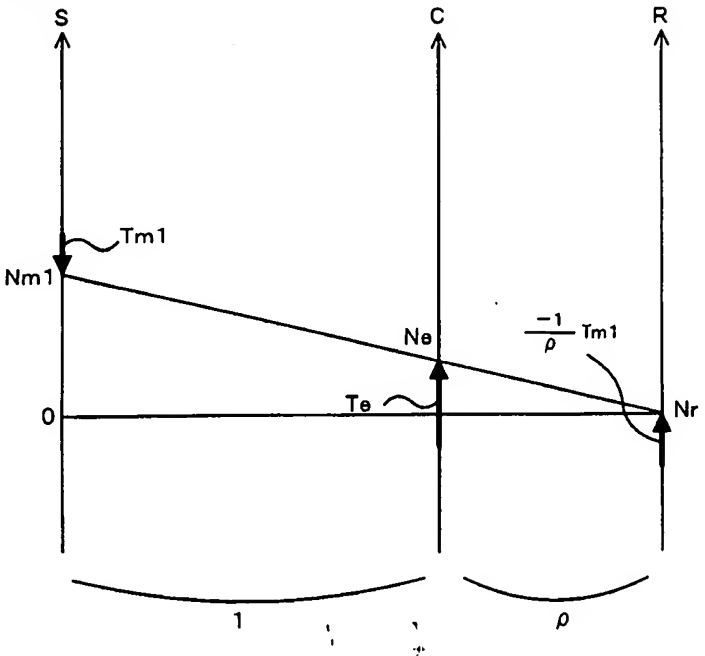
【図６】


154
154a
158
ハウジング側
154b
ベーン側
159
油圧

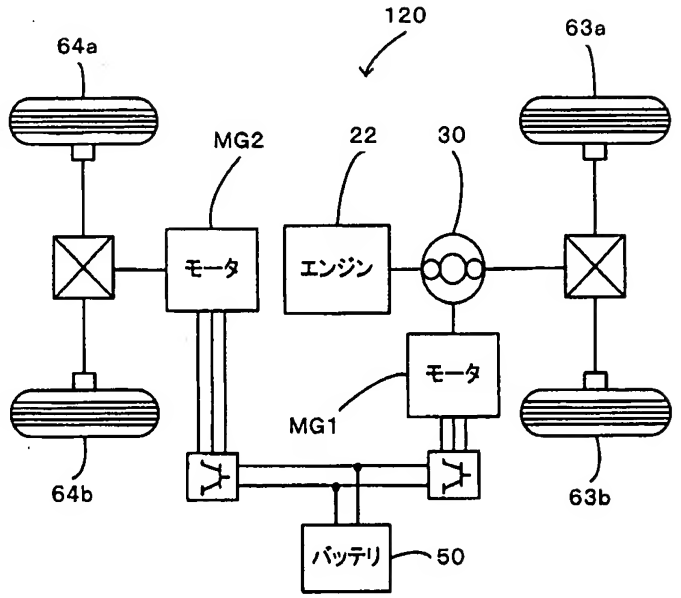
【図７】


アイドル要求時制御ルーチン
充電残量SOCの入力
S100
可変バルブタイミング機構を駆動制御
S110
S120
ロックピン解除？
NO
YES
S140
SOC＜S1？
NO
YES
S130
アイドル制御
エンジン回転数Ne=Neidl
S150
バッテリ充電制御
回転数Ne=Nech(>Neidl)
S160
エンジン停止
エンジン回転数Ne=0
RET

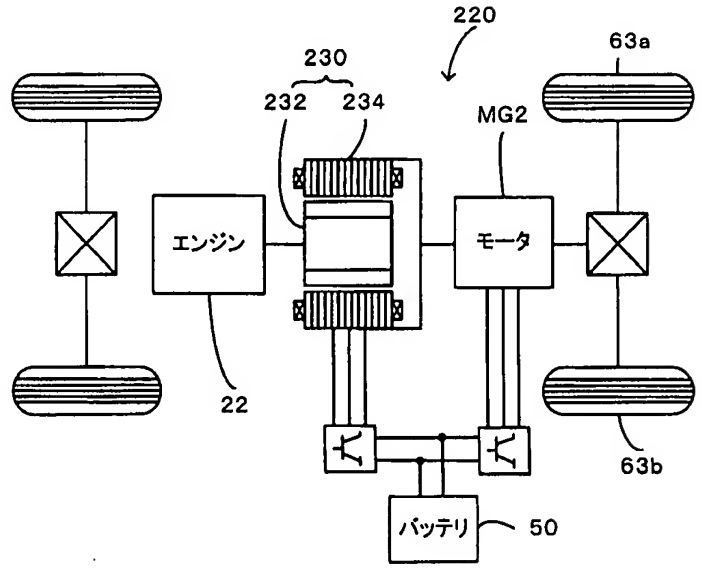
【図８】


S
C
R
Tm1
Nm1
Ne
−1/ρ Tm1
0
Te
Nr
1
ρ

【図９】


120
64a
63a
MG2
22
30
モータ
エンジン
モータ
MG1
64b
63b
バッテリ
50

【図１０】


220
230
232
234
63a
MG2
エンジン
モータ
22
63b
バッテリ
50